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# STANDARDS FOR DETERMINING THE PURITY OF MILK.

## THE LIMIT OF ERROR IN BACTERIOLOGICAL MILK ANALYSES.

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Anyone who has had any experience in comparative bacteriological tests knows that discrepancies in the reports of analyses of supposedly identical samples of milk are frequent, when the samples are submitted to different laboratories for examination. Sometimes it happens that the discrepancies reported are extremely wide, so wide, indeed, as to result in the grading of milk samples from the same bottle into different grades according to the laboratory at which the bacteriological analysis is made. These differences in the results of the analysis of identical samples have in some places led to a discrediting of the value of bacteriological analysis. That such discrepancies occur is beyond doubt; and the question arises whether they are due to inevitable difficulties in bacteriological analysis, whether bacteriological analysis has such inherent obstacles that it can not be made reliable, or whether they are due to differences in methods of technique in different laboratories sufficiently great to result in throwing the analyses widely apart. A further question then arises whether, if laboratories could be reduced to identical methods of technique, there would still remain divergence in results, and if so, how great the error of bacteriological milk analysis would be.

The American Public Health Association published some years ago its Standard Methods of Milk Analysis, which endeavored to formulate the methods that should be used in the bacteriological analysis of milk. These methods have since been regarded as *standard*, and it has been tacitly assumed that where bacteriological analysis has been made of milk the methods followed have been essentially the standard methods of the American Public Health Association. But in spite of this attempt to reach uniformity, divergent results of bacteriological analyses have continued to be repeated, and questions as to the reliability of all such work have been asked more and more insistently.

In October, 1914, four of the large laboratories in New York City determined upon a cooperative test by which some of these questions should if possible be answered. The laboratories concerned were the public health laboratory of the city of New York under the supervision of W. H. Park; the Lederle laboratory, the bacteriological work of which is directed by H. D. Pease; the sanitary laboratory of C. E. North; and the bacteriological laboratory of the Borden Condensed Milk Co., under the direction during the experiments first of Dr. F. X. Govers and later of Mr. W. D. Strack. These laboratories requested the author of this paper to serve as referee for the series of tests. The planning and coordinating of the whole series have been in his hands and all of the reports were forwarded to him for tabulation and study. This paper is the result of these tests, which have lasted seven months and involved something like 20,000 separate analyses by a variety of methods. It has not been possible to report in this paper all of these analyses, but the most significant ones are tabulated below.

The general plan of the experiments has been as follows: Upon certain convenient dates the referee distributed to the different laboratories a series of samples of milk, sometimes as many as 40, in other cases as few as 15. These samples represented milk of all types, pasteurized, raw, and of the various grades of milk sold in New York City, including certified as well as milk of grades A and B. They also included samples from other places that were not graded at all. As will be seen from the figures below, they represented, from a bacteriological standpoint, practically every type of milk that might reach the market, from the highest grade to the lowest. The samples were numbered in such a way that the laboratories could not compare notes with each other, and moreover, in each case the samples were frequently in duplicates. Sometimes each laboratory received three duplicate samples from the same bottle, all differently numbered, and in most cases two duplicate samples of each bottle were distributed. Of course, all of the laboratories received identical samples throughout, only under different numbers, and in the first series of tests each laboratory was to make the analysis of these samples by its ordinary methods of technique. All plates were to be counted at the same time—namely, after a period of 48 hours—and the report of each plate count was sent to the referee. The different laboratories were not expected to make their own interpretation of their figures, but were to leave that to the referee after the figures had been brought together and properly tabulated. The results of the figures after tabulation were returned to the laboratories, to serve them as guides in the next series of tests to be undertaken, and this plan was kept up through the whole of the first series of experiments. In the later series various modifications of the tests were made. As will be seen from the tables given below, quite a large number of factors which may be supposed to modify the results of bacteriological analysis were tested out in the different experiments, for the purpose of determining as closely as possible to what factors the discrepancies in the reports of the different laboratories were due.

## FIRST SERIES OF TESTS.

The first series of tests was by far the most extended and involved the analysis of 200 samples by each laboratory by five different methods and in three different dilutions, some 10,000 separate analyses in all. In this series the endeavor was first to determine how closely comparable the results of these four laboratories were when using methods of technique which each had adopted in its ordinary routine. For this purpose the samples to be tested were examined in the laboratories without informing the assistants who were making the analyses of the real significance of the work. The samples were then tested by the routine method in use in each laboratory at that time.

*The wire loop versus the pipette method of dilution.*—There had been developed in the laboratory of the New York Board of Health a method by which the use of pipettes might be dispensed with and in their place a platinum loop of a definite size, supposed to hold $\frac{1}{100}$ c. c. of milk, might be substituted. The accuracy of this method had never been tested out except in a single laboratory, and it was thought that possibly the use of the wire loop explained the fact that the board of health laboratory had the reputation of reporting higher results than other laboratories. In this series of tests similar loops were placed in the hands of each of the four laboratories, and the samples of milk to be tested were submitted to analysis both by the use of the wire loop and by the standard method of using pipettes. The routine of the board of health laboratory in the use of the wire loop was such as to furnish only a 100 and a 10,000 dilution, the 1,000 dilution being omitted; in the other laboratories a 1,000 dilution was also made.

*Media.*—To test out the question of whether the modification in media makes a noticeable difference in the results, three different media were adopted in this series of tests. One was the standard agar medium, as described in the standard methods. The second was the same as the standard but with the substitution of Liebig's beef extract for beef infusion. A third was a dilute medium made of beef extract, but with the nutritive ingredients reduced to one-twelfth of the amount that is present in the standard agar and having an acidity of only .3 per cent. These three were tested side by side in all of the first series of experiments.

The first series of tests consisted of five different test days. On each of these days there were submitted to the four laboratories 40 samples, variously duplicated and variously numbered, and each of them was tested by the three different media and the two different methods. This plan was repeated on five different days during a period of about three weeks, no attempt being made in this series to modify methods of routine in any of the laboratories.

*Effect of crowding.*—One general result of all of the series of tests here reported may be best mentioned at the outset. The more the plates are crowded with colonies the larger is the number of bacteria that are prevented from developing colonies, and therefore the lower is the count. In practically the whole series of thousands of experiments it was found that those plates in which the number of bacteria were over a thousand gave uniformly a lower proportional count than plates where the colonies were considerably under the 800 figure. In order to give a reliable result, the plates should contain a number of bacteria not over 200. It would even be desirable if the number of colonies should be between 40 and 200 on the plates that were to be counted; but this is possible only when miscellaneous samples of milk are examined by large numbers of separate dilutions, which in routine work seems to be out of the question. It must therefore be borne in mind that a high-seeded plate will give a marked underestimate of the total number of bacteria present in the milk sample.

*Explanatory remarks.*—In most of the following tables there is given only the final report which would be made as to the analysis. In order to show how this final figure has been obtained and also to show how the dilutions compare with each other, there are given in Table I the actual counts upon three illustrative samples just as they were received by the referee. In this table the final figure to be reported is in black-faced type, and in determining this figure the reports marked with an asterisk (*) were not used. In general the method of obtaining this final report was as follows:

No plates which contained less than 20 or more than 800 colonies were considered unless the milk had so few bacteria that no plate had as many as 20 or so many that even the 10,000 dilution gave more than 800 to a plate. Where two plates were found with colonies between 20 and 800 the final figure was an average of the two. This will be understood from the examination of Table I, in which the figures found with each dilution are given, as well as the final report, the latter marked "Rep."

In Table II and all following tables the different dilution figures are omitted, only the final report being given.

In all of the tables samples with the same number are samples from the same bottle of milk thoroughly mixed.

The different laboratories are represented by letters, and where two or more reports are given the same letter and number it indicates that two or more samples of the same bottle of milk were given to each laboratory under different labels.

"S. method" indicates the loop method and "P. method" the pipette method of securing $\frac{1}{100}$ c. c. for diluting.

"P. media" refers to the dilute media used (containing one-twelfth the standard amount of nutrients). "S. media" refers to the standard beef-infusion medium adjusted to 1.5 per cent acid. "Ex. media" refers to the standard medium made with beef extract instead of beef infusion.

After the number of the sample the letters indicate the character of the milk, "P." indicating pasteurized, "C." certified, and "R." raw milk.

The letter "S" in the tables indicates a spreader, and a (?) indicates that for some reason the count was not reliable. The sign (–) indicates that no report was sent.

"Unc." indicates that the colonies were so numerous that they were not counted.

TABLE I.—*A complete report upon three illustrative samples.*

SAMPLE NO. 3.

| | Dilution. | P. method. P. med. | P. method. S. med. | S. method. S. med. | S. method. P. med. | S. method. Ex. med. |
|---|---|---|---|---|---|---|
| Laboratory A | 100 | 500 | S. | 2,200 | 200 | 100 |
| | 1,000 | 2,000* | 3,000 | 2,000* | 2,000* | 7,000* |
| | 10,000 | 10,000* | 20,000* | (–) | 10,000* | (–) |
| | Rep. | **500** | **3,000** | **2,200** | **200** | **100** |
| Laboratory A | 100 | 700 | 3,900 | (–) | 500 | 1,000 |
| | 1,000 | (–) | 20,000* | 1,000 | 1,000* | (–) |
| | 10,000 | 10,000* | (–) | (–) | (–) | (–) |
| | Rep. | **700** | **3,900** | **1,000** | **500** | **1,000** |
| Laboratory B | 100 | 700 | 1,100 | 700 | 600 | 500 |
| | 1,000 | 1,000* | 1,000* | 1,000* | 4,000* | 1,000* |
| | 10,000 | (–) | (–) | 10,000* | (–) | (–) |
| | Rep. | **700** | **1,100** | **700** | **600** | **500** |
| Laboratory B | 100 | 5,100 | 6,700 | 7,500 | 6,800 | 6,100 |
| | 1,000 | 4,000* | 4,000* | 12,000* | 7,000* | 4,000* |
| | 10,000 | (–) | (–) | (–) | (–) | (–) |
| | Rep. | **5,100** | **6,700** | **7,500** | **6,800** | **6,100** |
| Laboratory C | 100 | 4,000 | S. | S. | 300 | 5,000 |
| | 1,000 | (–) | (–) | (–) | (–) | (–) |
| | 10,000 | (–) | (–) | (–) | (–) | (–) |
| | Rep. | **4,000** | S. | S. | **300** | **5,000** |
| Laboratory C | 100 | ............ | ............ | ............ | ............ | ............ |
| | 1,000 | ............ | ............ | ............ | ............ | ............ |
| | 10,000 | ............ | Missing. | ............ | ............ | ............ |
| | Rep. | ............ | ............ | ............ | ............ | ............ |
| Laboratory D | 100 | 4,300 | 800 | 1,500 | 4,600 | 600 |
| | 1,000 | 2,000* | 2,000* | 1,000* | 5,000* | (–) |
| | 10,000 | 20,000* | (–) | 20,000* | 20,000* | 20,000* |
| | Rep. | **4,300** | **800** | **1,500** | **4,600** | **600** |
| Laboratory D | 100 | 9,000 | 3,400 | S. | 16,400 | 900 |
| | 1,000 | 17,000* | 9,000* | 9,000 | 3,000* | 1,000* |
| | 10,000 | 20,000* | (–) | 40,000* | 10,000* | 10,000* |
| | Rep. | **9,000** | **3,400** | **9,000** | **16,400** | **900** |

SAMPLE NO. 42.

| | | | | | | |
|---|---|---|---|---|---|---|
| Laboratory A | 100 | 1,600 | 2,100 | 6,500 | 58,000 | 51,000 |
| | 1,000 | 12,000* | 6,000* | 34,000 | 33,000 | 38,000 |
| | 10,000 | 90,000* | 40,000* | 10,000* | 140,000* | 60,000* |
| | Rep. | **1,600** | **2,100** | **20,000** | **44,000** | **44,000** |
| Laboratory A | 100 | 11,100 | 11,200 | 39,000 | 46,000 | 56,000 |
| | 1,000 | 60,000 | 10,000* | 52,000 | 91,000 | 80,000 |
| | 10,000 | 100,000* | 40,000* | 50,000* | 50,000* | 50,000* |
| | Rep. | **35,000** | **11,200** | **45,000** | **68,000** | **68,000** |

## TABLE I.—*A complete report upon three illustrative samples*—Continued.

### SAMPLE NO. 42—Continued.

| | Dilution. | P. method. P. med. | P. method. S. med. | S. method. S. med. | S. method. P. med. | S. method. Ex. med. |
|---|---|---|---|---|---|---|
| Laboratory A | 100 | 20,000 | 1,500 | 12,300 | 40,000 | 9,000 |
| | 1,000 | 30,000 | (−) | 52,000 | 32,000 | 12,000 |
| | 10,000 | 100,000* | 10,000* | 40,000* | 20,000* | 30,000* |
| | Rep. | **25,000** | **1,500** | **32,000** | **36,000** | **10,000** |
| Laboratory B | 100 | 42,000 | 1,000 | 1,100 | 80,000 | 64,000 |
| | 1,000 | 4,000* | 2,000* | 4,000* | 75,000 | 85,000 |
| | 10,000 | 10,000* | 10,000* | 10,000* | (−) | 70,000* |
| | Rep. | **42,000** | **1,000** | **1,000** | **77,000** | **74,000** |
| Laboratory B | 100 | 64,000 | 900* | 18,000 | 40,000 | 24,000 |
| | 1,000 | 160,000 | 240,000 | 160,000 | 25,000 | 230,000 |
| | 10,000 | 40,000* | 80,000* | (−) | 100,000* | 10,000* |
| | Rep. | **114,000** | **240,000?** | **79,000** | **32,000** | **127,000** |
| Laboratory B | 100 | (−) | (−) | 200 | 14,000 | Con. |
| | 1,000 | (−) | (−) | 4,000* | 4,000* | 13,000 |
| | 10,000 | (−) | (−) | (−) | (−) | 60,000* |
| | Rep. | (−) | (−) | **200** | **14,000** | **13,000** |
| Laboratory C | 100 | 150,000 | 5,100 | S. | 126,000 | S. 96,000 |
| | 1,000 | (−) | (−) | (−) | (−) | (−) |
| | 10,000 | 80,000* | (−) | (−) | 120,000* | 10,000* |
| | Rep. | **150,000** | **5,100** | S. | **126,000** | **96,000** |
| Laboratory C | 100 | 100,000 | 5,700 | 14,400 | 99,600 | 102,000 |
| | 1,000 | (−) | (−) | (−) | (−) | (−) |
| | 10,000 | 250,000 | 130,000 | 40,000* | 310,000* | 210,000* |
| | Rep. | **100,000** | **67,000?** | **14,400** | **99,000** | **102,000** |
| Laboratory C | 100 | 146,000 | 10,400 | 9,000 | 140,800 | 104,000 |
| | 1,000 | (−) | (−) | (−) | (−) | (−) |
| | 10,000 | 60,000* | 60,000* | 20,000* | 60,000* | 50,000* |
| | Rep. | **146,000** | **10,400** | **9,000** | **140,000** | **104,000** |
| Laboratory D | 100 | 42,000 | 16,000 | 15,600 | 96,000 | 48,000 |
| | 1,000 | 96,000 | 34,000 | (−) | (−) | 23,000 |
| | 10,000 | 40,000* | 20,000* | (−) | (−) | 13,000* |
| | Rep. | **69,000** | **25,000** | **15,600** | **96,000** | **35,000** |
| Laboratory D | 100 | 100 | 500 | S. | 400 | 54,000 |
| | 1,000 | (−) | 1,000* | 72,000 | 1,000* | 85,000 |
| | 10,000 | 1,000 | (−) | (−) | (−) | 50,000 |
| | Rep. | **100?** | **500** | **72,000** | **400** | **54,000** |
| Laboratory D | 100 | 200 | 400 | 300 | 72,000 | 96,000 |
| | 1,000 | 1,000* | (−) | (−) | 80,000 | 82,000 |
| | 10,000 | (−) | 10,000* | (−) | 140,000* | 10,000 |
| | Rep. | **200** | **400** | **300** | **76,000** | **88,000** |

### SAMPLE NO. 59.

| | | | | | | |
|---|---|---|---|---|---|---|
| Laboratory A | 100 | 16,000 | 34,000 | 110,000 | 21,800 | 100,000 |
| | 1,000 | 44,000 | 280,000 | 350,000 | 174,000 | 58,000 |
| | 10,000 | 80,000* | 190,000* | 360,000* | 100,000* | 230,000* |
| | Rep. | **30,000** | **157,000** | **230,000** | **98,000** | **79,000** |
| Laboratory A | 100 | 340,000* | (−) | 206,000 | 23,000 | 33,000 |
| | 1,000 | 420,000 | (−) | 740,000 | 280,000 | 260,000 |
| | 10,000 | 2,400,000 | (−) | 990,000 | 350,000 | 390,000 |
| | Rep. | **1,400,000** | (−) | **860,000** | **315,000** | **325,000** |
| Laboratory B | 100 | 410,000* | 1,000,000* | 1,100,000* | 390,000* | 300,000* |
| | 1,000 | 500,000 | 1,920,000 | 1,360,000 | 1,500,000 | 1,280,000 |
| | 10,000 | 2,170,000 | 3,000,000 | 1,450,000 | 3,000,000 | 3,800,000 |
| | Rep. | **1,330,000** | **2,460,000** | **1,400,000** | **2,250,000** | **2,540,000** |
| Laboratory B | 100 | 116,000* | 100,000* | 510,000* | 300,000* | 217,000* |
| | 1,000 | 800,000 | 320,000 | 760,000 | 300,000 | 287,000 |
| | 10,000 | 2,000,000 | 1,640,000 | 400,000 | 290,000 | 400,000 |
| | Rep. | **1,400,000** | **980,000** | **580,000** | **295,000** | **343,000** |
| Laboratory C | 100 | (−) | (−) | (−) | (−) | (−) |
| | 1,000 | (−) | (−) | (−) | (−) | (−) |
| | 10,000 | 1,400,000 | 1,100,000 | 1,900,000 | 2,500,000 | 2,190,000 |
| | Rep. | **1,400,000** | **1,100,000** | **1,900,000** | **2,500,000** | **2,190,000** |
| Laboratory C | 100 | (−) | (−) | (−) | (−) | (−) |
| | 1,000 | (−) | (−) | (−) | (−) | (−) |
| | 10,000 | 2,450,000 | 3,700,000 | 2,630,000 | 2,500,000 | 2,060,000 |
| | Rep. | **2,450,000** | **3,700,000** | **2,630,000** | **2,500,000** | **2,060,000** |
| Laboratory D | 100 | Unc. | Unc. | Unc. | (−) | S. |
| | 1,000 | Unc. | 132,000 | S. (17) | 73,000 | 360,000 |
| | 10,000 | 880,000 | 160,000* | 40,000 | 50,000* | 230,000* |
| | Rep. | **880,000** | **132,000** | (?) | **73,000** | **360,000** |
| Laboratory D | 100 | Unc. | Unc. | S. | Unc. | (−) |
| | 1,000 | Unc. | 11,000* | 360,000 | 1,200,000* | 300,000 |
| | 10,000 | 2,000,000 | 1,600,000 | 310,000 | 1,070,000 | 260,000 |
| | Rep. | **2,000,000** | **1,600,000** | **335,000** | **1,070,000** | **280,000** |

TABLE II.—*The final report which would be given for each sample of milk submitted to each laboratory.*

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 1 P | A | 100 | 4,900 | 3,300 | 1,300 | 600 |
| | A | 2,700 | 700 | 12,000 | 5,900 | 300 |
| | B | 500 | 900 | 500 | 300 | 400 |
| | B | 1,600 | 4,200 | 1,100 | 1,300 | 900 |
| | C | 3,200 | 2,000 | 3,700 | 4,500 | 4,000 |
| | C | S. | 1,500 | 2,300 | 3,600 | 2,700 |
| | D | 2,000 | 200 | 200 | 4,000 | (?) |
| | D | 300 | 100 | 700 | 1,200 | 1,000 |
| 2 P | A | 2,000 | 1,000 | 1,500 | 1,800 | 2,500 |
| | A | (?) | 1,200 | 1,400 | 2,500 | 1,600 |
| | B | 2,300 | 200 | 5,500 | 2,000 | 4,000 |
| | B | 3,300 | 3,060 | 300 | 2,500 | 1,100 |
| | C | 6,000 | 1,400 | 1,600 | 5,000 | 2,500 |
| | C | 3,700 | 1,300 | S. | 5,200 | 3,000 |
| | D | 300 | 300 | 1,800 | 4,200 | 2,200 |
| | D | 1,700 | 1,000 | 1,000 | 2,800 | 1,600 |
| 3 P | A | 400 | 500 | 1,000 | 36,000 | 52,000 |
| | A | 5,000 | 8,600 | 5,700 | 5,100 | 3,300 |
| | B | 300 | 300 | 1,600 | 500 | 900 |
| | B | 1,000 | (–) | 800 | 200 | 600 |
| | C | 16,100 | 12,800 | 20,000 | 15,900 | 25,000 |
| | C | 6,000 | 4,500 | 3,600 | 3,600 | 5,500 |
| | D | (–) | S. | (?) | (?) | 3,400 |
| | D | 100 | 100 | 1,400 | 800 | 100 |
| 4 P | A | 100 | 4,500 | 4,600 | 2,200 | 3,200 |
| | A | 1,700 | 4,900 | 1,000 | 22,000 | 12,000 |
| | B | 1,400 | 1,100 | 1,200 | 1,100 | 1,200 |
| | B | 1,200 | 500 | (–) | 2,200 | 300 |
| | C | 2,900 | 1,100 | 2,000 | 1,500 | 2,000 |
| | C | 1,400 | 1,700 | 1,000 | 3,600 | 3,100 |
| | D | 1,300 | 1,000 | 700 | 2,400 | 1,200 |
| | D | 500 | 1,000 | 600 | 300 | 1,000 |
| 5 C | A | 300 | 1,100 | 1,200 | 1,200 | 600 |
| | A | 200 | 4,500 | 4,800 | 2,100 | 1,400 |
| | B | 900 | 3,900 | 4,100 | 1,100 | 2,400 |
| | B | 1,900 | 2,500 | 6,100 | 2,500 | 5,100 |
| | C | 3,200 | 6,500 | 6,700 | 5,700 | 8,100 |
| | C | 13,200 | 7,800 | (?) | 8,000 | 9,200 |
| | D | 2,600 | 2,700 | 5,200 | 8,700 | 3,600 |
| | D | 2,000 | 3,300 | 4,600 | 8,100 | 5,100 |
| 6 P | A | 200 | 8,000S | 1,400 | 2,600 | 700 |
| | A | 600 | 500 | 1,500 | 2,200 | 1,000 |
| | B | (?) | (?) | 200 | 800 | 500 |
| | B | (?) | (?) | 5,000 | 300 | 22,000 |
| | C | 4,500 | 2,400 | 4,300 | 3,200 | 3,800 |
| | C | 2,700 | 3,400 | 4,000 | 5,000 | 4,100 |
| | D | 200 | 3,000 | 100 | 2,300 | 1,700 |
| | D | 800 | 100 | 300 | 1,300 | 1,700 |
| 7 P | A | 300 | 800 | 1,800 | 2,500 | 900 |
| | A | 800 | S. | 300 | 1,300 | 700 |
| | B | (?) | (?) | 300 | 800 | 1,000 |
| | B | (?) | 500 | 1,900 | (?) | (?) |
| | C | 5,700 | 2,500S | 4,500S | 15,800 | 12,200 |
| | C | 14,900 | S. | S. | 9,800 | 10,500 |
| | D | 600 | 600 | S. | 200 | S. |
| | D | 300 | 400 | 400 | 3,000 | 1,700 |
| 8 P | A | 100 | 500 | 1,900 | 400 | 400 |
| | A | 100 | 800 | 1,000 | 1,600 | 800 |
| | B | 1,500 | 12,000 | 1,400 | 4,700 | 1,700 |
| | B | 200 | 900 | 8,400 | 9,100 | 6,700 |
| | C | 8,300 | 8,400 | 12,900 | 12,000 | 12,400 |
| | C | 4,200 | 4,000 | 3,000 | 4,500 | 4,200 |
| | D | 3,300 | (?) | 5,400 | 4,600 | 10,000 |
| | D | 900 | 7,000 | 11,200 | 10,400 | 5,700 |
| 9 P | A | 1,000 | 600 | 3,600 | 1,400 | 3,300 |
| | A | 800 | 100 | 1,200 | 600 | 900 |
| | B | 100 | 200 | 200 | 300 | 200 |
| | B | 500 | 300 | 8,000 | 400 | 100 |
| | C | 1,400 | 1,800 | 5,000 | 2,000 | 2,100 |
| | C | 3,800 | 2,400 | (?) | 3,600 | 4,800 |
| | D | 2,000 | 600 | 1,400 | 4,800 | 400 |
| | D | 600 | 1,200 | 1,000 | 4,200 | 400 |

TABLE II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 10 C | A | 3,700 | 3,900 | 6,900 | 1,800 | (—) |
| | A | 1,100 | 400 | 3,700 | 2,000 | 2,800 |
| | B | 3,800 | 4,100 | 4,500 | 1,000 | 1,700 |
| | B | 600 | 1,100 | 3,200 | 3,700 | 3,600 |
| | C | 2,300 | 7,200 | 5,000 | 2,500 | 8,500 |
| | C | 2,800 | 5,800 | 4,800 | 5,000 | 7,300 |
| | D | 5,200 | 700 | 6,600 | 1,500 | 3,700 |
| | D | 6,400 | 1,100 | 5,100 | 5,600 | 2,800 |
| 11 P | A | 200 | 400 | 1,800 | 1,400 | 2,300 |
| | A | 200 | 1,000 | 2,800 | 800 | 1,800 |
| | B | 4,000 | 1,800 | 400 | 2,500 | 800 |
| | B | 1,000 | 200 | 700 | 200 | 900 |
| | C | 4,000 | S. | 4,500 | 6,500 | 8,200 |
| | C | 12,600 | S. | 6,100 | 13,000 | 6,000 |
| | D | 700 | S. | 3,000 | S. | 1,200 |
| | D | 5,900 | 1,500 | 4,400 | 10,800 | 1,800 |
| 12 P | A | 300 | 1,300 | 1,300 | 900 | 500 |
| | A | 100 | 600 | 1,200 | 700 | 2,000 |
| | B | 300 | 100 | 300 | 1,500 | 500 |
| | B | Contam. | 300 | 600 | 200 | (?) |
| | C | 19,700 | 10,000 | 4,500 | 4,000 | 9,000 |
| | C | 10,900 | S. | S. | 12,500 | 8,500 |
| | D | 8,700 | S. | 2,800 | 10,900 | 2,500 |
| | D | 8,700 | 600 | 1,800 | 6,500 | 300 |
| 13 P | A | 100 | 1,200 | 2,000 | 2,500 | 5,300 |
| | A | 1,000 | 300 | 200 | 300 | 500 |
| | A | (?) | (?) | 100 | 700 | 600 |
| | B | 5,800 | 5,100 | 4,300 | 5,700 | 4,600 |
| | B | 2,600 | 900 | 900 | 500 | 800 |
| | B | 2,200 | 1,800 | 1,900 | 2,100 | 2,400 |
| | C | 500 | S. | 700 | 600 | 4,500 |
| | C | 3,600 | 300 | 2,800 | 2,700 | 3,200 |
| | C | 800 | 800 | 1,400 | 1,300 | 4,000 |
| | D | 3,700 | 900 | 2,300 | 3,600 | 1,000 |
| | D | 3,900? | 300? | 1,100 | 3,800 | 500 |
| | D | 2,200 | 300 | 200 | 500 | 900 |
| 14 P | A | (?) | 1,300 | 3,400 | 1,200 | S. |
| | A | 300 | 200 | 1,900 | 2,100 | 600 |
| | B | 600 | 700 | 500 | 1,200 | 1,600 |
| | B | 300 | 300 | 400 | 900 | 400 |
| | C | 7,800 | S. | S. | 9,500 | 3,600 |
| | C | 13,300 | S. | S. | 10,500 | 6,200 |
| | D | 7,100 | 3,000? | 6,300 | 11,200 | 4,100 |
| | D | 10,500 | 1,200 | S. | 11,100 | 2,000 |
| 15 P | A | 3,700 | 2,700 | 2,300 | 2,000 | 2,300 |
| | A | 400 | 900 | 1,600 | 3,000 | 2,400 |
| | B | 1,000 | 600 | 700 | 1,800 | 1,500 |
| | B | 2,000 | 700 | 3,500 | 1,400 | 200 |
| | C | 4,500 | 2,300 | 2,200 | 6,500 | 4,000 |
| | C | 6,000 | 1,400 | 1,600 | 5,000 | 2,500 |
| | D | 2,000 | 300 | 1,400 | 3,200 | 2,500 |
| | D | 700 | 400 | 1,900 | 2,800 | 2,200 |
| 16 P | A | (?) | 2,000 | (?) | 400 | 1,600 |
| | A | 400 | 300 | 700 | 700 | 900 |
| | B | 1,300 | 1,900 | 2,000 | 4,200 | 1,100 |
| | B | 1,600 | 800 | 1,100 | 800 | 8,500 |
| | C | 2,300 | 1,000 | S. | 3,400 | 2,800 |
| | C | 1,300 | S. | S. | 2,200 | 400 |
| | D | 1,600 | S. | S. | 3,700 | S. |
| | D | 2,500 | 500 | S. | 500 | S. |
| 17 P | A | 500 | (?) | 1,400 | 300 | 2,200 |
| | A | 200 | 300 | 500 | 100 | 1,700 |
| | B | 3,600 | 1,300 | (—) | 3,400 | (?) |
| | B | (?) | 4,700 | 1,800 | 1,500 | 2,900 |
| | C | 7,500 | 2,100 | 2,800 | 8,600 | 6,200 |
| | C | 6,200 | 2,100 | 1,500 | 6,500 | 4,600 |
| | D | 500 | (?) | 2,700 | 1,100 | 400 |
| | D | 500 | 300 | 800 | 600 | 200 |
| 18 P | A | 1,600 | 4,700 | 2,000 | 2,500 | 5,300 |
| | A | 2,300 | 1,200 | 1,000 | 400 | 2,500 |
| | B | 4,400 | 4,500 | 4,800 | 6,200 | 4,900 |
| | B | 1,400 | 3,700 | 2,000 | 2,800 | 2,200 |
| | C | 3,400 | 1,400 | 2,700 | 9,200 | 9,000 |
| | C | 8,400 | 900 | 1,500 | 9,700 | 11,200 |
| | D | 5,400 | 3,500 | 6,400 | 5,800 | 3,500 |
| | D | 4,500 | 3,100 | 3,200 | 8,000 | 2,600 |

TABLE II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 19 P | A | 700 | 1,500 | 1,600 | 500 | 1,500 |
|  | A | 1,300 | 2,300 | 4,200 | 200 | 1,400 |
|  | B | 1,300 | 1,400 | 2,300 | 900 | 1,400 |
|  | B | 500 | 500 | 500 | 200 | 600 |
|  | C | 3,000 | 2,900 | 4,000 | 7,800 | (—) |
|  | C | 7,000 | 4,400 | S. | 6,200 | 4,500 |
|  | D | 1,000 | 1,000 | 1,400 | 900 | 1,000 |
|  | D | 600 | 200 | 2,000 | 1,400 | 2,100 |
| 20 P | A | 300 | 1,500 | 800 | 750 | 700 |
|  | A | 200 | 2,100 | 2,500 | 700 | 5,400 |
|  | B | 500 | 1,000 | 1,500 | 700 | 1,700 |
|  | B | 500 | 900 | 700 | 500 | 400 |
|  | C | 5,600 | 2,100 | 2,600 | 5,000 | 5,200 |
|  | C | 6,000 | 3,000 | 2,500 | 6,400 | 5,400 |
|  | D | (?) | 100 | 100 | 300 | 800 |
|  | D | 500 | 300 | 1,200 | 3,200 | 200 |
| 21 P | A | 2,700 | 2,400 | 900 | 400 | 1,600 |
|  | A | 200 | 500 | 1,200 | 1,400 | 300 |
|  | B | 1,100 | 1,700 | 700 | 500 | 300 |
|  | B | 300 | 900 | 6,000 | 700 | 700 |
|  | C | 5,300 | 1,200 | 4,000 | 12,300 | 7,000 |
|  | C | 4,300 | 1,900 | 1,300 | 1,900 | 5,500 |
|  | D | 5,500 | 2,000 | 800 | 700 | 800 |
|  | D | 300 | 2,800 | 800 | 2,000 | 2,800 |
| 22 P | A | 1,200 | 1,200 | 3,300 | 2,600 | 3,400 |
|  | A | 500 | 4,100 | 3,800 | 1,400 | 1.500 |
|  | B | 700 | 1,100 | 900 | 600 | 700 |
|  | B | 700 | 900 | 1,300 | 900 | 1,000 |
|  | C | 5,900 | 4,000 | 6,200 | 7,100 | 7,600 |
|  | C | 6,700 | 4,700 | 5,900 | 6,500 | 8,300 |
|  | D | 600 | 300 | 1,900 | 1,200 | 1,700 |
|  | D | 900 | 1,100 | 2,500 | 2,500 | 900 |
| 23 P | A | 4,000 | 1,300 | 3,000 | 2,600 | 2,800 |
|  | A | (?) | 700 | 1,200 | 700 | 2,300 |
|  | B | 1,100 | 1,600 | 2,600 | 8,000 | 3,100 |
|  | B | (—) | 1,500 | 2,000 | 6,300 | 5,500 |
|  | C | 6,200 | 2,400 | 3,400 | 6,800 | 4,600 |
|  | C | 11,000 | 2,200 | 1,700 | 8,000 | 6,700 |
|  | D | (?) | 100 | 400 | 1,200 | 1,000 |
|  | D | 3,900 | 1,000 | 1,000 | 2,500 | 1,300 |
| 24 P | A | 4,000 | 1,100 | 500 | 800 | 300 |
|  | A | 100 | 300 | 1,800 | 2,100 | 1,700 |
|  | B | 6,600? | 7,200 | 800 | 400 | 400 |
|  | B | 500 | (—) | 600 | 400 | 400 |
|  | C | 9,700 | 10,400 | 15,200 | 1,200 | 10,400 |
|  | C | 8,700 | 1,500 | 1,400 | 6,700 | 5,400 |
|  | D | 1,500 | 100 | 900 | 2,000 | 1,500 |
|  | D | 500 | 500 | 800 | 2,200 | 1,000 |
| 25 P | A | 300 | 600 | 2,200 | 300 | 1,200 |
|  | A | 300 | 3,400 | 3,700 | 1,500 | 2,000 |
|  | B | 300 | (—) | 2,300 | 600 | 800 |
|  | B | 900 | 1,800 | 300 | 2,000 | 600 |
|  | D[1] | 2,000 | 2,400 | 3,000 | 2,000 | 2,000 |
|  | D[1] | 200 | 500 | 4,200 | 3,000 | 800 |
|  | D | 500 | 600 | 3,000 | 3,100 | 1,300 |
|  | D | 800 | 1,800 | 3,000 | 200 | 1,900 |
| 26 P | A | 100 | 1,000 | 3,700 | 500 | 400 |
|  | A | 200 | 3,500 | 2,700 | 200 | 1,600 |
|  | B | 10,000 | 22,000 | 4,400 | 1,400 | 4,900 |
|  | B | 1,800 | 2,500 | 3,500 | 2,300 | 3,400 |
|  | C | 9,000 | 5,800 | 4,800 | 4,300 | 6,200 |
|  | C | 8,500 | 1,400 | 6,500 | 4,500 | 6,000 |
|  | D | 700 | 4,700 | 5,800 | 700 | 900 |
|  | D | 600 | 3,400 | 3,800 | 800 | 1,200 |
| 27 C | A | 100 | 5,100 | 700 | 700 | 1,000 |
|  | A | 400 | 2,400 | 2,100 | 400 | 700 |
|  | B | 1,500 | 2,200 | 1,100 | 800 | 1,300 |
|  | B | 2,600 | 5,000 | 600 | 400 | 700 |
|  | C | 2,900 | 2,000 | 3,000 | 3,700 | 5,900 |
|  | C | 8,400 | 6,400 | 7,000 | 10,400 | 5,000 |
|  | D | 4,200 | 2,300 | 2,600 | 4,000 | 2,000 |
|  | D | 800 | 1,600 | 3,400 | 1,200 | 2,200 |

[1] By mistake sent to D instead of C.

TABLE II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 28 P | A | 500 | 11,000 | 1,600 | 600 | 900 |
| | A | 2,000 | 2,000 | 700 | 600 | 1,600 |
| | B | 1,400 | 1,100 | 1,200 | 1,100 | 1,200 |
| | B | 1,200 | 1,400 | 900 | 1,100 | 600 |
| | C | 3,200 | 100 | 1,200 | 4,000 | 3,200 |
| | C | 1,400 | 600 | 2,000 | 3,800 | 3,800 |
| | D | 4,300 | 800 | 1,500 | 4,600 | 600 |
| | D | 3,300 | 800 | 1,600 | 2,600 | 600 |
| 29 P | A | 500 | 1,200 | 100 | 100 | 4,000 |
| | A | 1,800 | 3,500 | 2,800 | 200 | 2,600 |
| | B | 1,800 | 1,300 | 1,700 | (–) | 2,000 |
| | B | 4,500 | 3,700 | 1,800 | 1,700 | 1,500 |
| | C | 2,300 | (–) | 2,200 | 4,400 | 5,700 |
| | C | 3,700 | 1,200 | 2,000 | 2,000 | 5,200 |
| | D | 3,100 | 1,600 | 1,700 | 1,200 | 1,900 |
| | D | 1,900 | 1,600 | 2,200 | 4,100 | 2,600 |
| 30 P | A | 100 | (?) | 300 | 600 | 3,000 |
| | A | (?) | (?) | (?) | 200 | 300 |
| | B | 3,000 | 1,700 | 4,000 | 700 | 600 |
| | B | 1,800 | 1,700 | 1,600 | 3,000 | 1,700 |
| | C | 1,400 | 1,000 | 400 | 4,100 | 5,000 |
| | C | 700 | 1,000 | S. | 1,800 | S. |
| | D | 2,700 | 1,500 | 6,000 | 14,800 | 700 |
| | D | 11,200 | 1,400 | 700 | 12,800 | 200 |
| 31 P | A | 500 | 3,000 | 2,200 | 200 | 100 |
| | A | 700 | 3,000 | 1,000 | 500 | 1,000 |
| | B | 700 | 1,100 | 700 | 600 | 500 |
| | B | 5,100 | 6,700 | 7,500 | 6,800 | 6,100 |
| | C | 4,000 | S. | S. | 300 | 5,000 |
| | C | ........... | Missing. | ........... | ........... | ........... |
| | D | 4,300 | 800 | 1,500 | 4,600 | 600 |
| | D | 9,000 | 3,400 | 9,000 | 16,400 | 900 |
| 32 C | A | 1,200 | 2,100 | 900 | 1,800 | 6,100 |
| | A | 3,000 | 400 | 2,700 | 200 | (–) |
| | B | 1,000 | 1,600 | 1,500 | 1,600 | 1,600 |
| | B | 1,100 | 1,500 | 4,100 | 1,100 | 1,700 |
| | C | 3,500 | 4,000 | 5,100 | 2,100 | 3,700 |
| | C | 1,800 | 2,000 | 4,300 | 2,000 | 4,200 |
| | D | 1,500 | 2,600 | 4,200 | 2,300 | 3,100 |
| | D | 900 | 3,000 | 3,500 | 3,700 | 1,800 |
| 33 C | A | 2,000 | 2,300 | 2,400 | 500 | 1,400 |
| | A | 500 | 2,000 | 4,300 | 1,400 | 1,300 |
| | B | 2,700 | 3,800 | 2,000 | 1,300 | 1,600 |
| | B | 1,000 | 1,700 | 4,200 | 4,600 | 5,500 |
| | C | 3,200 | 4,100 | 2,300 | 5,000 | 6,600 |
| | C | 900 | 3,200 | 2,300 | 2,200 | 4,600 |
| | D | 300 | 2,400 | 2,300 | 1,600 | 2,800 |
| | D | 1,300 | 2,000 | 3,600 | 3,200 | 2,800 |
| 34 C | A | 1,900 | 2,000 | 2,900 | (–) | 900 |
| | A | 2,000 | 2,400 | 1,500 | 1,000 | 400 |
| | B | 800 | 4,800 | 1,800 | 1,600 | 1,700 |
| | B | 1,500 | 2,500 | (–) | 1,900 | 2,200 |
| | C | 4,000 | 1,900 | 2,900 | 2,000 | 3,000 |
| | C | 1,300 | 1,000 | 3,600 | 1,400 | 2,500 |
| | D | 600 | 3,300 | 2,800 | 4,500 | 2,100 |
| | D | 1,600 | 3,000 | 3,000 | 3,600 | 2,600 |
| 35 P | A | 1,000 | 200 | 1,200 | 500 | 400 |
| | A | 3,300 | 14,000 | 7,800 | 6,700 | 8,000 |
| | B | 800 | 1,100 | 3,200 | 2,100 | 2,200 |
| | B | 40,000 | (–) | 1,400 | 3,800 | 700 |
| | C | 15,300 | 10,200 | (?) | 22,400 | 15,300 |
| | C | 5,300 | (?) | 9,500 | 13,200 | 11,000 |
| | D | 17,000 | 2,700 | 5,500 | 17,400 | 3,000 |
| | D | 1,200 | 600 | 7,000 | 12,100 | 18,300 |
| 36 R | A | 20,000 | 35,000 | 52,000 | 25,000 | 27,000 |
| | A | 29,000 | 74,000 | 49,000 | 28,000 | 21,000 |
| | B | 8,500 | 8,600 | 9,400 | 6,600 | 5,900 |
| | B | 25,000 | 35,000 | 20,000 | 16,000 | 29,000 |
| | C | 64,000 | 89,000 | 120,000 | 129,000 | 136,000 |
| | C | 86,000 | 94,000 | 95,000 | 108,400 | 94,000 |
| | D | 8,800 | 8,600 | 26,000 | 27,000 | 61,000 |
| | D | 46,000 | 26,000 | 33,000 | 55,000 | 59,000 |

TABLE II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 37 R | A | 20,000 | 60,000 | 42,000 | 23,000 | 25,000 |
| | A | 22,000 | 41,000 | 49,000 | 12,500 | 14,000 |
| | B | 23,000 | 22,000 | 17,000 | 15,000 | 20,000 |
| | B | .......... | .......... | 64,000 | 16,000 | 44,000 |
| | C | 90,000 | 100,000 | 108,000 | 96,000 | 102,000 |
| | D[1] | 95,000 | 115,000 | 47,000 | 48,000 | 54,000 |
| | D | 36,000 | 42,000 | 69,000 | 43,000 | 58,000 |
| | D | 44,000 | 72,000 | 72,000 | 40,000 | 33,000 |
| 38 R | A | 10,000 | 66,000 | 31,000 | 10,400 | 31,000 |
| | A | 8,300 | 34,000 | (?) | 32,000 | 41,000 |
| | B | 43,000 | 46,000 | 71,000 | 3,500 | 2,700 |
| | B | 36,000 | 34,000 | 14,000 | 16,000 | 28,000 |
| | C | 94,000 | 88,000 | 95,000 | 100,000 | 102,000 |
| | C | 96,000 | 91,000 | 92,000 | 106,000 | 103,000 |
| | D | 67,000 | 63,000 | 76,000 | 31,000 | 43,000 |
| | D | 60,000 | 63,000 | 61,000 | 20,000 | 42,000 |
| 39 R | A | 23,000 | 35,000 | 54,000 | 14,000 | 33,000 |
| | A | 56,000 | 86,000 | 53,000 | 48,000 | 88,000 |
| | B | 88,000 | 95,000 | 30,000 | 27,000 | 27,000 |
| | B | 128,000 | 29,000 | 102,000 | 95,000 | 71,000 |
| | C | 52,000 | 45,000 | 72,000 | 44,000 | 75,000 |
| | C | 200,000 | 107,000 | 250,000 | 90,000 | 200,000 |
| | D | 72,000 | 70,000 | 36,000 | 38,000 | 62,000 |
| | D | 65,000 | 66,000 | 114,000 | 44,000 | 76,000 |
| 40 R | A | 32,000 | 65,000 | 77,000 | 35,000 | 85,000 |
| | A | 1,100 | 58,000 | 38,000 | 4,100 | 23,000 |
| | B | 92,000 | 100,000 | 150,000 | 119,000 | 127,000 |
| | B | 71,000 | 36,000 | 115,000 | 105,000 | 69,000 |
| | C | 300,000 | 240,000 | 270,000 | 280,000 | 370,000 |
| | C | .......... | Missing. | .......... | .......... | .......... |
| | D | 60,000 | 57,000 | 14,000 | 90,000 | 95,000 |
| | D | 120,000 | 220,000 | 44,500 | 63,000 | 53,000 |
| 41 R | A | 31,000 | 50,000 | 31,000 | 7,900 | 18,000 |
| | A | 1,000? | 89,000 | 92,000 | 70,000 | 100,000 |
| | B | 92,000 | 74,000 | 115,000 | 122,000 | 69,000 |
| | B | 161,000 | 132,000 | 100,000 | 111,000 | 97,000 |
| | C | 560,000 | 180,000 | 240,000 | 360,000 | 370,000 |
| | C | 130,000 | 130,000 | 240,000 | 210,000 | 240,000 |
| | D | 75,000 | 55,000 | 27,000 | 44,000 | 38,000 |
| | D | 65,000 | 93,000 | 40,000 | 36,000 | 22,000 |
| 42 R | A | 30,000 | 71,000 | 19,000 | 14,500 | 48,000 |
| | A | 35,000 | 62,000 | 71,000 | 19,000 | 65,000 |
| | B | 14,600 | 43,000 | 67,000 | 76,000 | 61,000 |
| | B | 35,000 | 18,000 | 15,400 | 58,000 | 37,000 |
| | C | 44,000 | 57,100 | 98,400 | 95,000 | 106,900 |
| | C | 100,000 | 100,000 | 200,000 | 70,000 | 110,000 |
| | D | 49,000 | 54,000 | 6,000 | 5,700 | 59,000 |
| | D | 70,000 | 40,000 | (?) | (?) | 65,000 |
| 43 R | A | 41,000 | 54,000 | 83,000 | 29,000 | 82,000 |
| | A | 22,000 | 27,000 | 62,000 | 36,000 | 63,000 |
| | B | 78,000 | 90,000 | 227,000 | 78,000 | 151,000 |
| | B | 73,000 | 117,000 | 223,000 | 52,000 | 78,000 |
| | C | 370,000 | 560,000 | 450,000 | 700,000 | 600,000 |
| | C | 270,000 | 280,000 | 590,000 | 350,000 | 450,000 |
| | D | 160,000 | 410,000 | 230,000 | 122,000 | 203,000 |
| | D | 127,000 | 120,000 | 40,000 | 50,000 | 200,000 |
| 44 P | A | 1,700 | 21,000 | 20,000 | 16,000 | 13,200 |
| | A | 3,000 | 6,100 | 14,000 | 13,000 | 21,000 |
| | B | 20,000 | 120,000? | 11,000 | 70,000 | 30,000? |
| | B | 5,200 | 1,800 | 11,000 | 9,000 | 10,000 |
| | C | 29,000 | 39,000 | 42,000 | 24,000 | 48,000 |
| | C | 42,900 | 39,000 | 37,000 | 32,900 | 38,400 |
| | D | 19,100 | 20,000 | 20,000 | 11,500 | 19,700 |
| | D | 20,000 | 45,000 | 18,000 | 22,000 | 17,000 |
| 45 C | A | 10,000 | 6,500 | 13,000 | 3,800 | 11,800 |
| | A | 1,200 | 8,700 | 17,000 | 3,600 | 20,000 |
| | B | 5,000 | (—) | (—) | 4,800 | (—) |
| | B | 1,000 | (—) | (—) | (?) | 12,000 |
| | C | 36,200 | 29,400 | 22,400 | 20,000 | 28,500 |
| | C | 24,800 | 28,000 | 20,000 | 30,000 | 28,900 |
| | D | 2,300 | 8,400 | 12,400 | 11,400 | 14,900 |
| | D | 2,100 | 9,000 | 11,800 | 10,000 | 6,000 |

[1] By error analyzed by D instead of C.

Table II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 46 P | A | 13,000 | 17,000 | 38,000 | 30,000 | 26,000 |
| | A | 32,000 | 25,000 | 25,000 | 30,000 | 34,000 |
| | B | 20,000 | 2,300 | 23,000 | 62,000 | 28,000 |
| | B | 19,000 | 1,800 | 17,000 | 40,000 | 14,000 |
| | C | 73,600 | 22,000 | 34,200 | 71,200 | 45,500 |
| | C | 42,000 | 26,000 | 38,500 | 129,600 | 35,000 |
| | D | 330,000 | 17,300 | 28,000 | 21,000 | 31,000 |
| | D | 44,000 | 40,000 | 24,000 | 23,000 | 36,000 |
| 47 R | A | 1,600 | 2,100 | 20,000 | 44,000 | 44,000 |
| | A | 35,000 | 11,200 | 45,000 | 68,000 | 68,000 |
| | A | 25,000 | 1,500 | 32,000 | 36,000 | 10,000 |
| | B | 42,000 | 1,000 | 1,000 | 77,000 | 74,000 |
| | B | 114,000 | 240,000? | 79,000 | 32,000 | 127,000 |
| | B | (—) | (—) | 200 | 14,000 | 13,000 |
| | C | 150,000 | 5,100 | S. | 126,000 | 96,000 |
| | C | 100,000 | 67,000? | 14,400 | 99,000 | 102,000 |
| | C | 146,000 | 10,400 | 9,000 | 140,000 | 104,000 |
| | D | 69,000 | 25,000 | 15,600 | 96,000 | 35,000 |
| | D | 100? | 500 | 72,000 | 400 | 54,000 |
| | D | 200 | 400 | 300 | 76,000 | 88,000 |
| 48 R | A | 350,000 | 628,000 | 448,000 | 190,000 | 210,000 |
| | A | 104,000 | 452,000 | 580,000 | 520,000 | 370,000 |
| | B | 30,000 | 19,000 | 52,000 | 203,000 | 400,000 |
| | B | 76,000 | 11,000 | 184,000 | 118,000 | 381,000 |
| | C | 1,610,000 | 1,540,000 | 1,470,000 | 1,070,000 | 1,410,000 |
| | C | 2,410,000 | 2,330,000 | 1,450,000 | 1,430,000 | 1,800,000 |
| | D | 128,000 | 126,000 | 525,000 | 400,000 | 610,000 |
| | D | 720,000 | 248,000 | 330,000 | 240,000 | 550,000 |
| 49 R | D[1] | 48,000 | 80,000 | 66,000 | 45,000 | 36,000 |
| | A | 6,800 | 62,000 | 57,000 | 30,000 | 94,000 |
| | B | 8,200 | 6,600 | 24,000 | 17,000 | 28,000 |
| | B | 13,000 | 12,000 | 41,000 | 15,000 | 22,000 |
| | C | 121,200 | 102,000 | 126,000 | 147,000 | 132,000 |
| | C | 97,000 | 87,000 | 116,000 | 112,000 | 122,000 |
| | D | 201,000 | 130,000 | 81,000 | 71,000 | 70,000 |
| | A[2] | 25,600 | 50,000 | 99,000 | 24,000 | 50,000 |
| 50 R | A | 24,600 | 52,000 | 65,000 | 33,000 | 20,400 |
| | A | 15,000 | 51,000 | 52,000 | 39,000 | 48,000 |
| | B | 16,000 | 17,000 | 58,000 | 17,000 | 17,500 |
| | B | 7,700 | 17,400 | 26,600 | 10,600 | 54,000 |
| | C | 150,000 | 180,000 | 154,000 | 135,000 | 184,000 |
| | C | 50,000 | 137,000 | 166,000 | 133,000 | 100,000 |
| | D | 9,400 | 57,000 | 12,300 | 38,000 | 104,000 |
| | D | 34,000 | 52,000 | (?) | 59,000 | 70,000 |
| 51 R | A | 20,000 | 34,000 | 25,000 | 11,000 | 29,000 |
| | A | 31,000 | 49,000 | 73,000 | 18,000 | 14,000 |
| | B | 4,100 | 21,000 | 41,000 | 31,000 | 36,000 |
| | B | 7,800 | 20,000 | 14,000 | 13,200 | 18,600 |
| | C | 104,600 | 76,000 | 124,000 | 108,000 | 142,000 |
| | C | 105,000 | 107,000 | 100,000 | 107,400 | 111,000 |
| | D | 14,000 | 33,000 | 80,000 | 71,000 | 87,000 |
| | D | 105,000 | (?) | 100,000? | 61,000 | 73,000 |
| 52 R | A | 38,000 | 102,000 | 45,000 | 13,200 | 19,000 |
| | A | 15,000 | 45,000 | 54,000 | 34,000 | 53,000 |
| | B | 24,000 | 5,200 | 18,700 | 9,300 | 23,000 |
| | B | 7,900 | 13,900 | 72,000 | 19,000 | 39,000 |
| | C | 120,000 | 144,000 | 140,800 | 136,000 | 184,000 |
| | C | 112,000 | 109,000 | 109,000 | 104,000 | 120,000 |
| | D | 83,000 | 107,000 | 95,000 | 74,000 | 50,000 |
| | D | 92,000 | 112,000 | 91,000 | 95,000 | 47,000 |
| 53 R | A | 10,000 | 26,000 | 61,000 | 85,000 | 18,000 |
| | A | 36,000 | 40,000 | 55,000 | 17,300 | 81,000 |
| | B | 11,400 | 35,000 | 36,000 | 21,300 | 43,000 |
| | B | 16,000 | 48,000 | 48,000 | 32,000 | 50,000 |
| | C | 83,000 | 119,000 | 102,000 | 176,000 | 122,000 |
| | C | 131,000 | 97,000 | 109,000 | 106,000 | 146,000 |
| | D | 30,000 | 72,000 | 90,000 | 85,000 | 53,000 |
| | D | 113,000 | 180,000 | 121,000 | 81,000 | 40,000 |
| 54 R | A | 12,400 | 60,000 | 120,000 | 16,100 | 480,000? |
| | A | 10,000 | 50,000 | 94,000 | 24,000 | 50,000 |
| | B | 6,800 | 23,000 | 58,000 | 11,900 | 32,000 |
| | B | 11,300 | 28,000 | 30,000 | 19,000 | 13,700 |
| | C | 166,000 | 125,000 | 159,000 | 140,000 | 125,000 |
| | C | 226,000 | 168,000 | 206,000 | 116,000 | 131,000 |
| | D | 66,000 | 50,000 | 86,000 | 27,000 | 84,000 |
| | D | 24,000 | 78,000 | 92,000 | 77,000 | 74,000 |

[1] By error examined by D.

[2] By error examined by A.

**Table II.**—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 55 R | A | 23,000 | 51,000 | 75,000 | 66,000 | 84,000 |
| | A | 41,000 | 38,000 | 71,000 | 14,000 | 47,000 |
| | B | 11,300 | 64,000 | 495,000 | 308,000 | 290,000 |
| | B | 5,800 | 12,400 | 4,700 | 2,400 | 2,900 |
| | C | 58,000 | 120,000 | 116,000 | 109,000 | 162,000 |
| | C | 43,000 | 141,000 | 123,000 | 125,000 | 139,000 |
| | D | 48,000 | 72,000 | 106,000 | 82,000 | 43,000 |
| | D | 122,000 | 177,000 | 100,000 | 85,000 | 49,000 |
| 56 R | A | 34,000 | 51,000 | 58,000 | 31,000 | 44,000 |
| | A | 110,000 | 46,000 | 80,000 | 27,000 | 120,000 |
| | A | 41,000 | 41,000 | 42,000 | (—) | 50,000 |
| | B | 7,700 | 41,800 | 70,000 | 19,000 | 39,000 |
| | B | 8,900 | 14,400 | 43,000 | 17,800 | 45,000 |
| | B | 9,900 | 31,000 | 11,000 | 11,000 | 10,000 |
| | C | 230,000 | 202,000 | 200,000 | 95,000 | 170,000 |
| | C | 87,000 | 99,000 | 160,000 | 78,000 | 224,000 |
| | C | 202,000 | 115,000 | 260,000 | 155,000 | 260,000 |
| | D | 40,000 | 72,000 | 98,000 | 106,000 | 52,000 |
| | D | 6,000 | 52,000 | 75,000 | 19,000 | 80,000 |
| | D | 25,000 | 39,000 | 102,000 | 55,000 | 1[illegible],000 |
| 57 R | A | 42,000 | 436,000 | 350,000 | 190,000 | [illegible]0,000 |
| | A | 121,000 | 490,000 | 415,000 | 72,000 | [illegible]80,000 |
| | B | 100,000 | 41,000 | 120,000 | 200,000 | 320,000 |
| | B | 106,000 | 103,000 | 77,000 | 190,000 | 280,000 |
| | C | 2,190,000 | 1,660,000 | 1,410,000 | 1,340,000 | 1,830,000 |
| | C | 2,000,000 | 2,110,000 | 1,980,000 | 1,850,000 | 2,000,000 |
| | D | 560,000 | 300,000 | 530,000 | 350,000 | 440,000 |
| | D | 400,000 | 218,000 | 382,000 | 420,000 | 670,000 |
| 58 R | A | 34,000 | 450,000 | 650,000 | 81,000 | 153,000 |
| | A | 218,000 | 533,000 | 445,000 | 330,000 | 306,000 |
| | B | (—) | (—) | 64,000 | 35,000 | 80,000 |
| | B | 125,000 | 99,000 | 220,000 | 80,000 | 480,000 |
| | C | 1,240,000 | 2,000,000 | 1,210,000 | 1,530,000 | 1,740,000 |
| | C | 1,570,000 | 1,250,000 | 2,160,000 | 1,650,000 | 2,000,000 |
| | D | 77,000 | 71,000 | 136,000 | 480,000? | 160,000 |
| | D | 380,000 | 220,000 | 490,000 | 405,000 | 300,000 |
| 590 P | A | 30,000 | 157,000 | 230,000 | 98,000 | 79,000 |
| | A | 1,400,000 | (—) | 860,000 | 315,000 | 325,000 |
| | B | 1,330,000 | 2,460,000 | 1,400,000 | 2,250,000 | 2,540,000 |
| | B | 1,400,000 | 980,000 | 580,000 | 295,000 | 343,000 |
| | C | 1,400,000 | 1,100,000 | 1,900,000 | 2,500,000 | 2,190,000 |
| | C | 2,450,000 | 3,700,000 | 2,630,000 | 2,500,000 | 2,060,000 |
| | D | 880,000 | 132,000 | (?) | 73,000 | 360,000 |
| | D | 2,000,000 | 1,600,000 | 335,000 | 1,070,000 | 280,000 |
| 600 P | A | 250,000 | 62,000 | 72,800 | 40,000 | 66,000 |
| | A | 315,000 | 680,000 | 230,000 | 56,000 | 234,000 |
| | A | 130,000 | 1,250,000 | 2,500,000 | 1,200,000 | 1,500,000 |
| | B | 2,170,000 | 850,000 | 1,600,000 | 1,000,000 | 1,120,000 |
| | B | 1,000,000 | 1,140,000 | 2,400,000 | 160,000 | 1,800,000 |
| | B | 3,500,000 | 3,400,000 | 2,430,000 | 2,200,000 | 1,700,000 |
| | C | 2,800,000 | 900,000 | 1,600,000 | 2,920,000 | 2,410,000 |
| | C | 1,900,000 | 2,100,000 | 3,200,000 | 3,420,000 | 3,490,000 |
| | C | 2,800,000 | 2,400,000 | 1,140,000 | 3,800,000 | 2,400,000 |
| | D | 790,000 | 880,000 | 1,630,000 | 1,320,000 | 1,320,000 |
| | D | 480,000 | 125,000 | (?) | 23,000 | 3,700,000 |
| | D | 118,000 | (?) | 220,000 | 400,000 | 240,000 |
| 61 R | A | 14,000 | 251,000 | 580,000 | 420,000 | 600,000 |
| | A | .......... | Broken. | .......... | .......... | .......... |
| | B | 185,000 | 50,000 | 235,000 | 200,000 | 300,000 |
| | C | 1,330,000 | 1,400,000 | 1,460,000 | 1,080,000 | 1,410,000 |
| | C | 1,610,000 | 1,170,000 | 1,480,000 | 1,550,000 | 1,970,000 |
| | D | 750,000 | 710,000 | 600,000 | 420,000 | 510,000 |
| | D | 44,000 | 77,000 | 85,000 | 145,000 | 392,000 |
| 620 R | A | 4,500,000 | 6,750,000 | 8,100,000 | 7,500,000 | 6,500,000 |
| | A | 6,800,000 | 5,650,000 | 1,300,000 | 8,750,000 | 8,700,000 |
| | B | Error. | 1,000,000 | 3,200,000 | 8,000,000 | 5,600,000 |
| | B | 6,500,000 | 480,000 | 4,800,000 | 16,000,000 | 4,000,000 |
| | C | 35,840,000 | 38,720,000 | 38,720,000 | 26,080,000 | 39,520,000 |
| | C | 34,000,000 | 51,600,000 | 55,200,000 | 33,600,000 | 43,200,000 |
| | D | 6,000,000 | 6,000,000 | 4,800,000 | 4,800,000 | 4,800,000 |
| | D | 10,800,000 | (—) | 12,000,000 | 7,200,000 | (—) |

Table II.—*The final report which would be given for each sample of milk submitted to each laboratory*—Continued.

| No. | Laboratory. | L. method, P. media. | L. method, S. media. | S. method, S. media. | S. method, P. media. | S. method, Ex. media. |
|---|---|---|---|---|---|---|
| 630 R | A | 9,000,000 | 19,000,000 | 20,000,000 | 9,500,000 | 6,000,000 |
| | A | 8,500,000 | 12,200,000 | 13,750,000 | 18,900,000 | 18,000,000 |
| | B | 9,600,000 | 2,250,000 | 5,000,000 | 1,600,000 | 6,400,000 |
| | B | 4,000,000 | 3,900,000 | 2,000,000 | 56,000,000 | 8,000,000 |
| | C | 68,400,000 | 79,200,000 | 85,600,000 | 75,600,000 | 67,200,000 |
| | C | 96,000,000 | 105,000,000 | 120,000,000 | 118,000,000 | 118,800,000 |
| | D | (?) | (?) | (?) | (?) | 18,000,000 |
| | D | (?) | (?) | 18,000,000 | (?) | 7,200,000 |
| 640 R | A | 5,400,000 | 21,800,000 | 28,000,000 | 12,000,000 | 5,800,000 |
| | A | 15,800,000 | 7,200,000 | 16,200,000 | 40,800,000 | 14,200,000 |
| | B | 8,600,000 | 9,400,000 | 10,200,000 | 7,800,000 | 10,400,000 |
| | B | 6,600,000 | 7,000,000 | 6,300 000 | 3,600,000 | 8,800,000 |
| | C | 65,000,000 | 54,000,000 | 58,320,000 | 58,800,000 | 63,000,000 |
| | C | 56,000,000 | 44,800,000 | 52,000,000 | 54,000,000 | 64,800,000 |
| | D | 24,000,000 | 15,000,000 | 15,000,000 | 15,000,000 | 24,000,000 |
| | D | 9,000,000 | 12,000,000 | (–) | 9,600,000 | 9,000,000 |
| 650 R | A | 940,000 | 2,960,000 | 4,800,000 | 500,000 | 410,000 |
| | A | 710,000 | 7,100,000 | 7,300,000 | 2,000,000 | 740,000 |
| | A | 2,000,000 | 9,800,000 | 6,900,000 | 2,400,000 | 3,200,000 |
| | B | 3,400,000 | 3,700,000 | 700,000 | 720,000 | 430,000 |
| | B | 2,900,000 | 4,200,000 | 5,000,000 | 1,310,000 | 1,800,000 |
| | B | 1,500,000 | 4,000,000 | 760,000 | 426,000 | 750,000 |
| | C | 1,700,000 | 27,000,000 | 31,000,000 | 30,000,000 | (–) |
| | C | 25,800,000 | 31,600,000 | 35,000,000 | 28,000,000 | 30,000,000 |
| | C | 32,000,000 | 48,000,000 | 40,000,000 | 32,000,000 | 45,000,000 |
| | D | 870,000 | 9,000,000 | 7,200,000 | 7,200,000 | 6,000,000 |
| | D | 2,000,000 | 1,800,000 | (?) | 1,800,000 | 6,000,000 |
| | D | 24,000,000 | 15,000,000 | 18,000,000 | 4,800,000 | (?) |
| 660 R | A | 10,800,000 | 6,400,000 | 9,600,000 | 6,400,000 | 7,400,000 |
| | A | 20,000,000 | 1,200,000 | 320,000 | 8,200,000 | 12,000,000 |
| | B | 2,900,000 | 4,200,000 | 3,000,000 | 1,300,000 | 2,900,000 |
| | B | 4,200,000 | 4,000,000 | 3,000,000 | 3,700,000 | 3,800,000 |
| | C | 80,000,000 | 51,000,000 | 57,000,000 | 55,120,000 | 55,000,000 |
| | C | 64,000,000 | 70,400,000 | 80,320,000 | 40,000,000 | 62,720,000 |
| | D | 6,000,000 | 6,000,000 | 5,400,000 | 4,200,000 | 3,000,000 |
| | D | 5,400,000 | 5,400,000 | 4,800,000 | 6,000,000 | 6,000,000 |
| 670 R | A | 1,640,000 | 4,000,000 | 1,000,000 | 240,000 | 1,080,000 |
| | A | 2,000,000 | 7,000,000 | 9,100,000 | 3,100,000 | 4,000,000 |
| | B | (–) | (–) | 1,800,000 | 390,000 | 1,000,000 |
| | B | 650,000 | 2,000,000 | 3,300,000 | 1,000,000 | 1,450,000 |
| | C | 40,000,000 | 48,000,000 | 56,000,000 | 37,000,000 | 38,000,000 |
| | C | 23,000,000 | 32,000,000 | 26,000,000 | 21,000,000 | (–) |
| | D | 12,000,000 | 18,000,000 | 18,000,000 | 2,470,000 | (–) |
| | D | 3,000,000 | 10,200,000 | 15,000,000 | 12,000,000 | 3,000,000 |
| 680 S | A | 4,050,000 | 6,200,000 | 4,550,000 | 1,210,000 | 1,680,000 |
| | A | 3,400,000 | 8,000,000 | 4,400,000 | 4,600,000 | 4,700,000 |
| | B | 1,840,000 | 440,000 | 320,000 | 1,900,000 | 1,360,000 |
| | B | 1,500,000 | 800,000 | 550,000 | 6,700,000 | 5,400,000 |
| | C | 8,440,000 | 9,440,000 | 8,760,000 | 10,640,000 | 11,400,000 |
| | C | 10,800,000 | 6,300,000 | 7,850,000 | 10,800,000 | 7,600,000 |
| | D | 6,600,000 | 4,800,000 | 3,600,000 | 4,200,000 | 3,600,000 |
| | D | 9,000,000 | 7,200,000 | 6,600,000 | (–) | (–) |
| 690 S | A | 5,400,000 | 3,680,000 | 5,600,000 | 4,200,000 | 6,800,000 |
| | A | 3,400,000 | 11,000,000 | 7,600,000 | 7,200,000 | 4,100,000 |
| | B | 4,800,000 | 4,200,000 | 6,160,000 | 7,200,000 | 6,000,000 |
| | B | 7,880,000 | 3,000,000 | 1,700,000 | 2,360,000 | 4,200,000 |
| | C | 65,000,000 | 99,000,000 | 90,000,000 | 68,000,000 | 86,000 000 |
| | C | 66,000,000 | 90,000,000 | 85,000,000 | 45,000,000 | 58,000,000 |
| | D | 12,000,000 | 9,600,000 | 7,200,000 | 9,600,000 | 9,600,000 |
| | D | 42,000,000 | (–) | (–) | 10,800,000 | (–) |
| 700 S | A | 64,000,000 | 13,400,000 | 10,200,000 | 14,000,000 | 9,600,000 |
| | A | 3,800,000 | 9,600,000 | 40,000,000 | 8,000,000 | 6,200,000 |
| | B | 6,680,000 | 14,600,000 | 11,000,000 | 7,700,000 | 12,600,000 |
| | B | 5,600,000 | 3,600,000 | 2,200,000 | 6,400,000 | 2,600,000 |
| | C | 169,700,000 | 189,000,000 | 165,000,000 | 149,200,000 | 189,000,000 |
| | C | 160,000,000 | 160,000,000 | 156,000,000 | 160,000,000 | 170,000,000 |
| | D | 15,000,000 | (–) | 12,000,000 | 12,000,000 | 24,000,000 |
| | D | (–) | (–) | (–) | (–) | 90,000,000 |

## Analysis of the Results of the Above Tables.

*Grade of accuracy in results of analysis.*—That discrepancies in the results of analyses of samples of milk from the same bottle, analyzed by different laboratories, should occur is to be expected. It must be recognized that bacteriological analyses, since they deal with discrete, solid objects, can not be compared with chemical analyses, where solutions are concerned. Bacteria can not be distributed through the milk with absolute uniformity, and, moreover, they have a tendency to clump, so that two samples from the same bottle can hardly ever be alike in bacterial content. Besides these primary differences there must be recognized irregularities in technique, which may add to the variation in results. No extensive series of experiments such as these has been before carried out to determine how great irregularities must be expected.

A glance at the above tables emphasizes the occurrence of irregularities, and indeed they seem to be greater than most would have anticipated. Nearly any one of the tests will show these irregularities, and they are so evident that there is no need to call attention to any special instances. From these irregularities two general conclusions must be reached.

1. Under the ordinary conditions of laboratory analyses, results of different laboratories in analyzing two different samples from the same bottle of milk can not be relied upon as giving any close approximation to accuracy.

2. Single analyses of a milk sample can not be relied upon. Not only is every laboratory liable to make some unrecognized slip in technique, so as to give a single widely divergent result, but even where there are no palpable errors, individual results occasionally diverge noticeably from the general average. Hence, no milk analysis should be reported upon except as the result of the average of two or more tests. If four or five analyses are made, the results may be regarded as moderately accurate; but under ordinary conditions of laboratory work, discrepancies are so great that reliance should not be placed upon a single plate count.

### FACTORS PRODUCING VARIATIONS.

*Variations in medium.*—The Standard Methods of Milk Analysis places great emphasis on the exact composition and method of making the culture media, and far less emphasis on the manipulation of the specimen and the general technique of laboratory methods. The three different media used in this series of tests are sufficient to determine whether variations in media had any considerable effect upon the result. A cursory glance at the tables fails to show that any one of these media gives uniformly a higher or a lower count than the others, and to determine the actual result it is necessary, therefore, to resort to a careful comparison of averages. This was done as follows: Each test was calculated separately, the figures obtained by all of the laboratories with each milk sample, when tested upon each of the three media, being added together and the sum divided by the number of tests made of each sample. This gave the average count obtained by all of the laboratories by the use of each one of the three media. The figures thus obtained were compared to see which medium gave the highest count for the whole series combined, the assumption being, of course, that the medium that gave the highest count was, other things being equal, the best. For all of the tests in which analyses from all the laboratories were complete enough to make a fair calculation, the results were as follows:

In 30 samples the standard medium gave the highest count.

In 27 milk samples the dilute standard medium gave the highest count.

In 20 samples the extract medium gave the highest count.

By this method of calculation the standard medium was thus shown to be slightly superior to the others.

A different attempt to determine the value of the media was made by averaging the total numbers of bacteria found in each of the media. In making this calculation, samples of less than 10,000 bacteria were calculated by themselves, since manifestly one or two of the results which were calculated in millions added to these would outweigh the significance of the lower figures. By this method the results were as follows:

Average of all samples below 10,000 by the standard medium, 3,158.

Average of all samples below 10,000 by the dilute medium, 4,042.

Average of all samples below 10,000 by the extract medium, 3,980.

By this calculation the dilute medium gave the highest average and the standard the lowest.

The figures of the plates giving numbers in tens and in hundreds of thousands were so divergent that an average would be misleading and consequently was not made. An average of the counts of all giving figures of over 1,000,000 resulted about the same for the standard and the dilute medium, with a slightly higher average for the extract medium.

From these tests it is clear that, so far as numbers are concerned, no one medium has any decided advantage over another. Any differences that were due to the media were vastly less than those that were due to other factors. Evidently the variations in the result in the above tables were not primarily due to differences in composition of the media, but must have been due to some differences in technique used in the four laboratories or to inherent variations in samples.

It should be further noticed that the dilute medium above tested had an acid reaction of only 0.3, which is much below that of the reaction adopted for the standard media. Inasmuch, however, as this dilute medium gave practically the same counts as those of the other media, it is evident that a variation in the acidity between 0.3 and 1.5 makes practically no difference in the results.

*Spreaders.*—One further conclusion from the use of these media was noticeable. The size of the colonies which grow in the dilute media was considerably smaller than those in the standard media, making these plates possibly a little harder to count. On the other hand, there was very much less difficulty experienced in the appearance of spreaders. In these tests, 128 spreaders were reported from plates made with standard medium, 23 from plates made with the dilute medium, and 21 from the plates made with the extract. In the reduction of spreaders, therefore, the dilute medium and the extract medium seem to be decidedly superior to the standard medium made from beef infusion.

### COMPARISON OF THE RESULTS OF THE DIFFERENT LABORATORIES WITH EACH OTHER.

The total numbers reported by each laboratory upon each sample of milk were added together and the sum divided by the number of separate analyses made of each sample. This gave the average number of bacteria per c. c. reported from each laboratory for each sample of milk. In this calculation the figures obtained by the standard method only were used. The results of these analyses are as follows:

Laboratory C gave counts which emphatically exceeded the other laboratories, while laboratory B in most cases gave the lowest counts. In actual figures:

Laboratory C gave the highest counts in all but 11 samples.
Laboratory D gave the highest count in 7 samples.
Laboratory A gave the highest count in 3 samples.
Laboratory B gave the highest count in 1 sample only.
Laboratory B gave the smallest count in 59 samples.
Laboratory A gave the smallest count in 34 samples.
Laboratory D gave the smallest count in 8 samples.

In no instance did laboratory C give the lowest count.

The counts of the laboratories were in general in the order B, A, D, C, and this order was repeated over and over again. Out of 96 samples, comprising all in which this calculation could be fairly made, this order was repeated 47 times, and it therefore represented the average order of these laboratories.

In order to show the relation of the laboratories to each other in this series of tests Table III has been prepared. In this table there are given the average figures that each laboratory obtained from each of the samples of milk submitted, and in the fifth column is given the extent of the variation found in the reports of the different laboratories when analyzing unknown duplicate samples of milk. This variation was obtained by dividing the highest figure reported by any laboratory by the lowest figure reported by any laboratory upon duplicate samples. In this table the figures are arranged in five sets corresponding to five successive test days, and they include some of the analyses that were not listed in Table II. In some cases this variation was found to be almost unbelievably great.

TABLE III.—*Averages obtained by each laboratory upon each sample tested in the three media by standard method, arranged in regular series according to the reports from laboratory A.*

| Laboratory A. | Laboratory B. | Laboratory C. | Laboratory D. | Variation. | Laboratory A. | Laboratory B. | Laboratory C. | Laboratory D. | Variation. |
|---|---|---|---|---|---|---|---|---|---|
| 950 | 1,350 | 2,780 | 980 | 2.9 | 2,100 | 4,590 | 5,200 | 1,230 | 2.4 |
| 975 | 850 | 1,300 | 950 | 1.4 | 2,660 | 900 | 6,930 | 1,800 | 7.7 |
| 1,150 | 2,770 | 2,250 | 1,900 | 2.4 | 24,400 | 19,300 | 80,000 | 53,000 | 4.1 |
| 1,600 | 2,250 | 1,150 | 2,330 | 2 | 29,000 | 22,500 | 99,000 | 45,500 | 4.4 |
| 1,850 | 700 | 7,710 | 2,700 | 7.7 | 33,600 | 14,700 | 115,000 | 43,500 | 7.8 |
| 1,875 | 3,820 | 4,970 | 1,810 | 2.7 | 49,000 | 29,000 | 75,000 | 52,500 | 2.6 |
| 2,100 | 1,975 | 7,375 | 8,020 | 4 | 1,350,000 | 7,850,000 | 58,000,000 | 12,000,000 | 42.9 |
| 2,550 | 3,850 | 15,100 | 2,400 | 6.3 | 2,350,000 | 930,000 | 22,000,000 | 4,000,000 | 24.6 |
| 2,700 | 2,175 | 7,580 | 3,180 | 3.4 | 3,080,000 | 1,500,000 | 35,000,000 | 14,000,000 | 23.3 |
| 3,080 | 3,760 | 10,700 | 4,120 | 2.8 |  |  |  |  |  |
| 3,325 | 1,200 | 5,750 | 4,780 | 4.9 | 1,010 | 5,330 | 8,130 | 7,880 | 7.4 |
| 3,375 | 3,375 | 18,200 | 5,780 | 5.3 | 1,780 | .......... | 7,400 | 2,720 | 4.2 |
| 3,900 | 2,100 | 7,210 | 4,420 | 3.2 | 1,830 | 1,530 | 3,500 | 2,030 | 1.9 |
| 4,850 | 3,980 | 7,910 | 6,050 | 2 | 1,830 | 2,550 | 7,900 | 7,350 | 4.3 |
| 5,050 | 1,975 | 5,750 | 5,100 | 2.9 | 3,450 | 2,950 | 5,510 | 4,210 | 1.8 |
| 27,700 | 29,500 | 73,300 | 55,850 | 2.6 | 3,900 | 750 | 3,460 | 1,420 | 5.2 |
| 42,000 | 20,000 | 77,300 | 76,500 | 3.8 | 4,100 | 2,230 | 14,300 | 8,880 | 6.4 |
| 46,200 | 30,000 | 101,000 | 88,000 | 3.3 | 7,500 | 1,200 | 2,200 | 1,200 | 6.2 |
|  |  |  |  |  | 17,200 | 760 | 12,300 | 900 | 22.6 |
| 760 | 2,960 | 2,725 | 2,100 | 3.4 | 28,300 | 25,600 | 115,000 | 58,600 | 4.4 |
| 880 | 1,930 | 2,820 | 5,880 | 6.7 | 35,600 | 13,300 | 138,000 | 101,000 | 3.8 |
| 1,000 | 1,010 | 3,000 | 1,910 | 3 | 36,100 | 30,100 | 132,000 | 75,300 | 4.4 |
| 1,160 | 3,700 | (?) | 5,500 | 5 | 42,300 | 30,500 | 145,000 | 54,200 | 4.6 |
| 1,340 | 1,840 | 2,760 | 3,100 | 2.3 | 53,300 | 38,300 | 108,700 | 78,300 | 2.3 |
| 1,630 | 1,740 | 3,585 | 2,285 | 2.2 | 54,600 | 24,500 | 124,000 | 66,000 | 5 |
| 1,830 | 5,500 | 7,800 | 1,860 | 4.3 | 71,100 | 45,000 | 133,000 | 61,400 | 2.9 |
| 2,130 | 2,000 | 3,500 | 2,300 | 1.7 | 130,000 | 27,400 | 146,000 | 73,300 | 5.9 |
| 2,130 | 2,960 | 2,725 | 2,100 | 1.3 | 8,850,000 | 4,650,000 | 72,000,000 | 11,000,000 | 15.4 |
| 2,285 | 3,810 | 7,210 | 3,400 | 3.4 | 14,600,000 | 7,000,000 | 164,000,000 | 12,000,000 | 23.4 |
| 2,440 | 2,320 | 3,560 | 3,100 | 1.5 |  |  |  |  |  |
| 6,610 | 3,200 | 3,860 | 2,710 | 2.4 | 1,100 | 620 | 7,600 | 6,100 | 12.2 |
| 39,200 | 53,000 | 113,000 | 39,900 | 3 | 1,250 | 1,586 | 9,650 | 1,325 | 7.7 |
| 45,500 | 137,000 | 306,000 | 59,600 | 6.7 | 1,810 | 1,375 | 7,380 | 4,400 | 5.4 |
| 52,600 | 55,000 | 121,900 | 61,000 | 2.3 | 1,840 | 1,000 | 7,450 | 4,575 | 7.4 |
| 53,000 | 1,016,000 | 415,000 | 51,000 | 8 | 1,875 | 1,500 | 3,900 | 1,600 | 2.6 |
| 59,100 | 135,000 | 523,000 | 180,000 | 8.8 | 2,050 | 2,560 | 2,880 | 2,260 | 1.4 |
| 318,000 | 1,230,000 | 2,290,000 | 350,000 | 7.1 | 2,600 | 3,430 | 7,300 | 3,460 | 2.8 |
| 6,720,000 | 1,600,000 | 2,700,000 | 690,000 | 4 | 11,560 | 8,000 | 24,890 | 11,080 | 3.1 |
|  |  |  |  |  | 16,200 | 23,500 | 38,800 | 18,030 | 2.3 |
| 720 | 3,500 | 3,600 | 2,700 | 5 | 25,100 | 57,000 | 55,000 | 67,500 | 2.6 |
| 800 | 610 | 6,900 | 2,560 | 8.6 | 30,500 | 30,600 | 58,600 | 27,100 | 2.1 |
| 930 | 1,710 | 5,260 | 1,350 | 5.6 | 40,400 | 198,000 | 1,730,000 | 498,000 | 43.2 |
| 1,000 | 3,100 | 3,000 | 5,200 | 5.2 | 40,700 | 46,300 | 78,400 | 48,800 | 1.9 |
| 1,030 | 3,200 | 5,000 | 930 | 5.3 | 337,000 | 158,000 | 1,700,000 | 257,000 | 4.4 |
| 1,200 | 500 | 6,700 | 1,400 | 13.4 | 386,000 | 226,000 | 1,639,000 | 442,000 | 7.3 |
| 1,200 | 4,550 | 5,200 | 1,230 | 4.3 | 533,000 | 244,000 | 1,600,000 | 360,000 | 6.5 |
| 1,500 | 3,310 | 5,381 | 2,200 | 3.5 | 1,430,000 | 13,160,000 | 97,100,000 | Unc. | (?) |
| 1,580 | 980 | 5,000 | 1,700 | 5.4 | 3,500,000 | 2,700,000 | 8,190,000 | 4,500,000 | 3.3 |
| 1,810 | 1,160 | 2,500 | 2,080 | 2.2 | 8,500,000 | 53,000,000 | 39,000,000 | 6,000,000 | 7.3 |

### VARIATION FACTOR IN EACH LABORATORY.

An endeavor was made to determine the range of variation in each laboratory when analyzing duplicate samples of milk. To obtain this figure the highest number reported by each laboratory upon duplicate samples was divided by the lowest number reported. Thus if the figures agreed exactly, as they should in theory, the result would be 1, while if one analysis of duplicate samples gave twice as high a figure as the others, the variation figure would be 2. Evidently, the smaller the variation figure the more uniform are the results of the analysis of the duplicate samples. The average variation figures of all of the samples analyzed by each laboratory were then added together and the sum was divided by the number of samples reported upon, the result giving what may be called the average laboratory variation. In determining this average variation certain reports were excluded which were manifestly due to laboratory errors. The results of all of those which were included were as follows:

Average variation in laboratory A, second test day, *4.6.
Average variation in laboratory B, second test day, 2.6.
Average variation in laboratory C, second test day, 3.1.
Average variation in laboratory D, second test day, 7.7.
Average variation in laboratory A, third test day, 5.9.
Average variation in laboratory B, third test day, 3.2.
Average variation in laboratory C, third test day, 2.2.
Average variation in laboratory D, third test day, 3.7.
Average variation in laboratory A, fourth test day, 5.2.
Average variation in laboratory B, fourth test day, 2.7.
Average variation in laboratory C, fourth test day, 1.5.
Average variation in laboratory D, fourth test day, 2.9.
Average variation in laboratory A, fifth test day, 3.5.
Average variation in laboratory B, fifth test day, 6.1.
Average variation in laboratory C, fifth test day, 2.4.
Average variation in laboratory D, fifth test day, 3.7.

Combining all of these figures together so as to give a general average for each laboratory for the whole series of reports so far as they were complete, the following result was obtained:

General average variation of laboratory A, 4.8.
General average variation of laboratory B, 3.6.
General average variation of laboratory C, 2.1.
General average variation of laboratory D, 4.5.

The large variation shown in some cases was due to one or two widely divergent results. If these had been excluded, the several laboratories would have been much closer together. This variation of nearly 500 per cent in some cases, is certainly wider than would have been expected, and wider than should be permissible. It is manifestly desirable to determine if possible the cause of the variations.

### THE PLATINUM LOOP VERSUS THE PIPETTE METHOD OF DILUTION.

Without going into details, it may be stated that the figures in Table II show that the results obtained by the loop method were slightly more irregular than those obtained by the pipette method.

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* The first test day is omitted from this list, since the data are incomplete.

The variations in duplicate samples made by the loop method were somewhat larger than those made by the standard method of dilution by pipette; the differences, however, are not very great, and are less than the irregularities arising from other factors. The general conclusion to be reached from this test is that the loop method is less regular than the pipette method, and that diluting the milk samples by means of a pipette should be adhered to as a standard method rather than the somewhat shorter method of using a measured platinum loop. It is evident, however, that the difference between the results obtained by using the pipette and the loop are not sufficient to explain the uniformly larger results reported by the laboratory C. The loop did not give results either uniformly larger or smaller than the pipette method, but simply more irregular.

### CAUSES OF VARIATIONS.

It is quite evident from the above tables that there must be some differences in technique used by the different laboratories that will explain the divergencies in the results thus given. The practical uniformity in the figures obtained from the use of widely divergent media shows that the differences are not due to the media, and it hence follows that they must be due either to differences in samples sent to the different laboratories or to differences in technique. The next step in this series of tests was to visit the different laboratories and to watch the method of manipulation adopted in each in the handling of the milk samples. It became apparent at once after these visits were made that there were at least two differences in technique which might explain the irregularities, at least to a certain extent.

1. The Standard Methods of Milk Analysis recommends 25 shakes of the milk sample before dilution. It was found that there was considerable variation both in the number of shakes and in the violence of the shaking in these four laboratories.
2. The method of counting was found to be quite different in the four laboratories.

In the Standard Methods, under the directions for counting, is the following sentence: "Colonies too small to be seen with the naked eye or with a slight magnification shall not be considered in the count." That the exact meaning of this sentence is difficult to determine is shown by the fact that the four laboratories concerned in this test had interpreted it in four different ways. In one laboratory no magnifying power was used; in the second a magnifying power was used occasionally; in the third a magnifying power of 2½ diameters was used for all plates; and in the fourth a magnifying power of 3½ diameters was used for some plates, but not for others. Although it was insisted by some engaged in these tests that in most cases the naked eye could see as many colonies as the microscopic lens, it is perfectly evident that this would not uniformly be the case. By the use of the naked eye alone persons who are nearsighted will get a higher count when small colonies are concerned than those who are farsighted, and when the plates are highly seeded the attempt to count the colonies without a lens will surely give a different count from one made with a magnifying power.

There were certain other slight differences in laboratory technique that did not seem to be of any special significance.

## SECOND SERIES OF TESTS.

The object of the second series of tests was so far as possible to avoid the recognized sources of discrepancy in the first series, for the purpose of seeing whether the wide variations that had appeared in the first series would continue to appear when the methods of manipulation in the laboratory were brought more nearly to a uniform basis. In this second series also it was determined to make a test of two or three other factors which it was thought possibly might contribute to the accuracy of the result. The second test was planned as follows:

### Plan for Tests.

1. *Uniformity of shaking.*—To insure as close a uniformity in shaking as possible, a visit was made to each laboratory, and a demonstration was made by the referee to the assistants who were performing the work, to show them exactly the amount and the vigor of shaking to be adopted in this series of tests. It was stipulated that each sample and each dilution bottle should be shaken 25 times. In each case the shake was to be a double shake, in which the bottle was to pass through a distance of at least a foot.

2. *Care in following out technique.*—In the first series of tests the laboratories were expected to follow out their ordinary routine without change; in the second series the assistants in each laboratory were carefully informed about the scope and purpose of the test, and were given to understand that the accuracy of their work was under observation. It was thought that this would bring about in some cases a more careful attention to the details of the technique, and thus reduce irregularities that might otherwise creep in.

3. *Method of counting.*—Each of the laboratories in question was instructed to obtain an engravers' lens magnifying 3½ diameters, and was directed to count all plates by the use of this lens.

4. *Method of inoculating the plates.*—Three different methods of plate inoculation were in use in these laboratories. One laboratory inoculated test tubes containing 10 c. c. of melted agar with 1 c. c. of

the diluted milk and then rolled the tubes between the hands, finally pouring the whole into a petri dish. Others placed the 1 c. c. of diluted milk into a petri dish and poured melted agar upon it, subsequently gently agitating the whole to distribute the milk uniformly over the plate. In some cases the agar was poured from test tubes each containing 10 c. c., while in other cases the same method was used except that the melted agar was poured from a flask instead of from test tubes, the eye of the assistant being trusted to obtain about 10 c. c. of agar in each plate. The advantage of this method over that of using test tubes is chiefly in the line of saving in time and the use of glassware; though not recognized in the Standard Methods it has become quite widely used in routine laboratories. To test the accuracy of these methods each laboratory used the first and third of these above described, and two laboratories used also the second.

5. *Check on the accuracy of counting.*—In order to determine whether the differences in the counts were actually differences in the method or in accuracy of counting, in the second series of tests each plate was counted by two laboratories. After the plates had been counted by the first laboratory they were carried over to a second, and immediately counted in that laboratory; the reports of the two counts were then sent to the referee without conference between the laboratories concerned. This it was thought would determine the personal error in counting.

For this series of tests a smaller number of samples were submitted to the different laboratories, 15 in all being furnished, but these 15, as will be seen from the tables, contained a wide variety of milk samples, ranging from those with a very low bacteria count to those with the bacteria in millions.

The following table gives a condensed summary of the results obtained by the series of tests. In this table the same method of determining the final report was used as in the first series, namely, plates containing less than 20 and more than 900 bacteria per c. c. being excluded in the final result, except in cases where these plates alone could be used. The "A method" referred to in the tables indicates the inoculation of the plates by the dilution with the agar poured directly into the plates from flasks. The "C method" was the same except that the agar was poured from tubes, and the "B method" was the method of inoculating the tubes with the diluted milk, rolling them and subsequently pouring the whole into the petri dish. It should be stated also that though this table contains the most significant data, it does not contain all of the data obtained in the test. The first three columns in all cases were the counts made by the laboratory that made the plates, the second three columns the counts of the second laboratory to which the same plates were submitted.

TABLE IV.

| | Counted by laboratory— | A method. | B method. | C method. | Counted by laboratory— | A method. | B method. | C method. |
|---|---|---|---|---|---|---|---|---|
| 71 R..... | A | 4,600 | 3,000 | 2,300 | C | 4,800 | 2,400 | 9,200 |
| | A | 5,100 | 5,400 | 3,200 | C | 1,200 | 4,000 | 5,000 |
| | B | 800 | 400 | (–) | D | 600 | 200 | (–) |
| | B | 1,100 | 4,100 | (–) | D | 300 | (?) | (–) |
| | C | 4,000 | 8,000 | 13,200 | A | 3,200 | 7,300 | 10,700 |
| | C | 11,000 | 10,000 | 3,500 | A | 3,500 | 10,900 | 15,000 |
| | D | 4,300 | 2,800 | (–) | B | 5,700 | 3,400 | (–) |
| | D | 2,000 | 2,100 | (–) | B | 1,400 | 2,600 | (–) |
| 72 P...... | A | 29,000 | 26,000 | 24,000 | C | 28,000 | 24,500 | 28,000 |
| | A | 15,000 | 16,700 | 10,000 | C | 22,000 | S. | 13,500 |
| | B | 1,300 | 7,700? | (–) | D | 100 | 6,500 | (–) |
| | B | 3,100 | 36,400? | (–) | D | 300 | 100 | (–) |
| | C | 11,000 | 16,500 | 20,200 | A | 16,200 | 15,700 | 23,300 |
| | C | 18,000 | 12,500 | 15,300 | A | 18,500 | 9,600 | 29,000 |
| | D | 4,900 | 40,000 | (–) | B | 3,000 | 36,000 | (–) |
| | D | 35,000 | 29,000 | (–) | B | 30,000 | 33,000 | (–) |
| 73 C...... | A | 3,100 | 2,700 | 2,900 | C | 2,400 | 3,500 | 2,600 |
| | A | 2,700 | 2,700 | 2,400 | C | 2,300 | 2,200 | 3,000 |
| | B | 2,100 | 3,800 | (–) | D | 800 | 1,200 | (–) |
| | B | 4,500 | 3,300 | (–) | D | 2,700 | 1,500 | (–) |
| | C | 3,000 | 8,000 | 3,000 | A | 3,400 | 3,600 | (–) |
| | C | 2,500 | 1,500 | 2,500 | A | 2,700 | 1,600 | 3,000 |
| | D | 3,000 | 2,800 | (–) | B | 10,900 | 3,600 | (–) |
| | D | 2,300 | 2,500 | (–) | B | 2,300 | 2,600 | (–) |
| 74........ | A | 38,000 | 36,000 | 44,000 | C | 37,000 | 53,000 | 54,000 |
| | A | 39,500 | 34,000 | 38,000 | C | 37,000 | 45,000 | 43,000 |
| | B | 100,000 | 101,000 | (–) | D | 65,000 | 105,000 | (–) |
| | B | 93,000 | 133,000 | (–) | D | 55,000 | 86,000 | (–) |
| | C | 47,000 | 510,000 | 380,000 | A | 36,000 | 231,000 | 353,000 |
| | C | 44,000 | 360,000 | 314,000 | A | 56,000 | 500,000 | 500,000 |
| | D | 32,000 | 220,000 | (–) | B | 26,000 | 38,000 | (–) |
| | D | 260,000 | 229,000 | (–) | B | 246,000 | 263,000 | (–) |
| 75 R..... | A | 3,200,000 | 2,920,000 | 5,000,000 | C | 3,800,000 | 4,000,000 | 5,600,000 |
| | A | 6,280,000 | 4,800,000 | 4,030,000 | C | 5,500,000 | 5,800,000 | 6,800,000 |
| | B | 8,750,000 | 6,020,000 | (–) | D | 6,600,000 | 6,000,000 | (–) |
| | B | 7,520,000 | 19,460,000 | (–) | D | (–) | 12,600,000 | (–) |
| | C | 3,300,000 | 3,200,000 | 3,600,000 | A | 4,600,000 | 4,200,000 | 4,050,000 |
| | C | 6,400,000 | 4,260,000 | 5,200,000 | A | 4,400,000 | 2,570,000 | 4,400,000 |
| | D | 2,630,000 | 2,330,000 | (–) | B | 2,560,000 | 2,300,000 | (–) |
| | D | 3,150,000 | 2,050,000 | (–) | B | 2,860,000 | 3,330,000 | (–) |
| 76 R..... | A | 5,340,000 | 5,760,000 | 5,800,000 | C | 8,800,000 | 5,600,000 | 5,800,000 |
| | A | 6,200,000 | 7,000,000 | 7,100,000 | C | 5,900,000 | 6,900,000 | 5,000,000 |
| | B | 15,540,000 | 14,560,000 | (–) | D | 6,600,000 | 6,600,000 | (–) |
| | B | 18,550,000 | 17,850,000 | (–) | D | 13,200,000 | 15,000,000 | (–) |
| | C | 10,800,000 | 10,000,000 | 10,000,000 | A | 11,750,000 | 13,600,000 | 10,800,000 |
| | C | 8,400,000 | 7,000,000 | 8,600,000 | A | 6,400,000 | 7,250,000 | 6,200,000 |
| | D | 7,200,000 | 8,810,000 | (–) | B | 9,900,000 | 9,240,000 | (–) |
| | D | 9,600,000 | 8,400,000 | (–) | B | 11,220,000 | 9,240,000 | (–) |
| 77 R..... | A | 450,000 | 352,000 | 410,000 | C | 320,000 | 360,000 | 470,000 |
| | A | 380,000 | 300,000 | 360,000 | C | 325,000 | 470,000 | 340,000 |
| | A | 370,000 | 400,000 | 360,000 | C | 435,000 | 510,000 | 300,000 |
| | B | 1,000,000 | 901,000 | (–) | D | 500,000 | 430,000 | (–) |
| | B | 448,000 | 1,640,000 | (–) | D | 313,000 | 841,000 | (–) |
| | B | 690,000 | 1,160,000 | (–) | D | 440,000 | 580,000 | (–) |
| | C | 475,000 | 510,000 | 410,000 | A | 587,000 | 480,000 | 490,000 |
| | C | 520,000 | 520,000 | 460,000 | A | 670,000 | 325,000 | 565,000 |
| | C | 350,000 | 260,000 | 350,000 | A | 505,000 | 600,000 | 340,000 |
| | D | 319,000 | 380,000 | (–) | B | 440,000 | 570,000 | (–) |
| | D | 317,000 | 342,000 | (–) | B | 390,000 | 400,000 | (–) |
| | D | 234,000 | 260,000 | (–) | B | 220,000 | 260,000 | (–) |

## Summary of the General Conclusions of the Second Series of Tests.

### UNIFORMITY OF THE MEDIA.

Samples of the media used by each laboratory were submitted to the referee for examination. These samples were tested first for acidity and were found to agree in acidity to within 0.1 per cent, three of the four media giving exactly 1 per cent acidity and the other 0.9 per cent acid. Each of the four media was then tested by the referee on the same samples of milk. Two samples of raw milk were diluted with 100 c. c. of water and shaken more thoroughly than usual, so as to give as perfect distribution of bacteria as possible. These were then plated in triplicate plates with each of the three media. The average counts of the three plates gave the following results:

| | Laboratory A. | Laboratory B. | Laboratory C. | Laboratory D. |
|---|---|---|---|---|
| No. 1 | 12,000 | 15,000 | 14,000 | 13,000 |
| No. 2 | 305,000 | 290,000 | 280,000 | 279,000 |

These results showed that the four samples of media were uniform and gave practically identical results, the variations being only those that were inevitable in examination of different samples of the same milk. The figures may also serve to show how close results may be obtained upon a single sample of milk when media and methods are absolutely uniform and when an average of three plates of each sample is made.

### COMPARISON OF DIFFERENT METHODS OF INOCULATION.

The figures in this series of tests showed a very slight superiority of the method of inoculating the tubes directly over the more common method of inoculating plates. The actual results were as follows:

In 30 separate tests the direct plate inoculation gave higher figures.

In 29 separate tests the tube method of inoculation gave the higher figures.

Averaging the total tests of each milk sample, however, showed that the tube inoculation gave the highest counts in five of the milk samples and the direct inoculation in one sample. Among this series of samples was one of cream, and this gave a considerably wider variation in results when the plate was inoculated directly over those obtained when the tubes were inoculated. Only two of the laboratories used the method of inoculating the petri dishes and subsequently pouring the agar from test tubes, so that the results are hardly extensive enough to be of much significance. So far as they go, the inoculation of the plates and the pouring of the plates from flasks gave higher results than the plate inoculation poured from tubes, this result being obtained in 15 samples, while the reverse result was obtained in 5 samples.

The very slight superiority of inoculating tubes directly is hardly sufficient to make it necessary to insist upon the adoption of this method in the place of the common method of inoculating the plate directly, where the latter is preferred. The slightly higher counts are less than the errors due to the irregular counting of the same plates in different laboratories. Where a large amount of routine work is done the saving in labor in the use of flasks rather than test tubes is so great as to make this method preferable, if it is not manifestly unreliable. The result of this series of tests shows that the two methods are so close to each other that no insistance should be made upon the method advised by the present Standard Methods of Milk Analysis, and it would seem to be wise to make it permissible to inoculate the petri dish from flasks rather than from test tubes.

### UNIFORMITY OF RESULTS.

The results of this series of tests were far more uniform than those of the first series, and may be briefly summarized as follows:

Each of the four laboratories reported four of the samples submitted as containing less than 10,000 bacteria.

Each of the laboratories reported two of the samples as containing between 500,000 and 20,000.

Each of the laboratories reported three samples as containing between 200,000 and 1,000,000, and, with the exception of one report from one laboratory, as containing between 200,000 and 500,000.

Each of the laboratories reported four of the samples as containing above 2,000,000 and less than 20,000,000.

A careful study of all the figures of which the above table is a summary also shows that in general the single analyses of the samples of milk agreed within these limits, and that the above statement applies not simply to averages, but also to individual analyses of separate samples.

The following table gives a condensed summary of the results in a form to make them more easily comparable to each other:

TABLE V.

| Numbers | Laboratory. | Average. | General average. | Extremes. |
|---|---|---|---|---|
| 73...... | A | 3,930 | 4,235 | 2,300- 5,400 |
| | B | 1,350 | | 400- 4,100 |
| | C | 8,850 | | 3,500- 13,200 |
| | D | 2,800 | | 2,000- 4,300 |
| 71...... | A | 2,750 | 3,300 | 2,700- 3,100 |
| | B | 3,425 | | 2,100- 4,500 |
| | C | 3,410 | | 1,500- 8,000 |
| | D | 2,650 | | 2,300- 2,800 |
| 72...... | A | 20,100 | 20,700 | 10,000- 29,000 |
| | B | 12,120 | | 1,300- 36,400 |
| | C | 23,400 | | 11,000- 20,200 |
| | D | 27,200 | | 4,900- 35,000 |
| 74[1]..... | A | 38,200 | 185,000 | 34,000- 44,000 |
| | B | 106,700 | | 93,000- 133,000 |
| | C | 413,000 | | 44,000- 510,000 |
| | D | 185,000 | | 32,000- 260,000 |
| 77...... | A | 375,000 | 521,000 | 300,000- 450,000 |
| | B | 974,000 | | 448,000-16,400,000 |
| | C | 428,000 | | 250,000- 520,000 |
| | D | 308,700 | | 234,000- 380,000 |
| 75...... | A | 6,550,000 | 7,440,000 | 2,920,000- 6,280,000 |
| | B | 10,440,000 | | 6,020,000-19,460,000 |
| | C | 4,400,000 | | 3,200,000- 6,400,000 |
| | D | 2,540,000 | | 2,050,000- 3,150,000 |
| 76...... | A | 6,200,000 | 10,000,000 | 5,340,000- 7,100,000 |
| | B | 16,600,000 | | 14,500,000 18,500,000 |
| | C | 9,130,000 | | 7,000,000-10,800,000 |
| | D | 8,500,000 | | 7,200,000- 9,600,000 |

[1] No 74 was a sample of cream.

*Individual variations.*—Isolated individual variations have not been wholly eliminated from this series of results, but they are less noticeable than in the previous tests. The individual variations do not appear in the summary table given above, but are very evident in the whole series of figures as presented. In some cases they were clearly due to errors in counting, for it was manifest that occasional dirt specks in the media were counted as colonies by one laboratory, whereas the second laboratory counting the same plates did not count them at all. It was also noticeable that most of the large individual variations were from the reports on the samples of milk that contained few bacteria. This is easily explained by the inevitable variation in the uniformity of distribution of bacteria through the milk.

*Comparison of the results of the four laboratories.*—In this series of tests no one of the laboratories gave a uniformly higher count than the others, as was the case in the first series. The almost uniform order in which the laboratories reported their results in the previous series was not shown in this series at all.

The analyses reported by the four laboratories upon this series of samples were very much more uniform than in the first series. They not only agreed more closely with each other, but the different reports from the same laboratory upon duplicate samples of milk agreed more closely. Although what has been termed in a preceding page the "average variation" (obtained by dividing the highest result by the lowest in each sample) ranged from two up to six in the first series, in this series the average variations in the different laboratories were, laboratory A, 1.8, laboratory B, 1.9, laboratory C, 2.2, laboratory D, 1.6. In obtaining these figures, however, one or two quite divergent results were omitted from two of the laboratories, since they were manifestly errors in counting; but even if they were included the results would be very much closer than in the previous series.

### VARIATIONS IN THE COUNTING OF DUPLICATE PLATES.

Interesting data are found by comparing the duplicate counts made by the different laboratories of identical plates. It is practically never that the duplicate counts agree, and in some cases the discrepancy was as great as the irregularities arising from all other sources. There was clearly a difference in the method adopted in counting the plates; in some laboratories the whole plates being counted, in others only a part counted and the whole estimated. An estimation of the number of colonies was naturally made, however, only where the plates were very heavily seeded. The details of these duplicate counts were as follows:

Laboratory A and C counted each other's plates, and in this case laboratory A obtained the higher count in 48 plates and laboratory C the higher count in 39 plates.

When counting the plates of laboratory C, laboratory A obtained in 53 plates a higher count than that reported by laboratory C.

When counting plates of laboratory A, laboratory C obtained in 34 plates a count higher than that reported by laboratory A.

In both cases each laboratory obtained a smaller count on its own plates in a larger number of instances than it obtained a larger count.

Laboratory B and laboratory D exchanged plates. In this series laboratory B obtained a higher count in 49 plates, laboratory D a higher count in 9 plates.

In only one set did laboratory D report higher counts than laboratory B upon the same plates.

In 73 cases the second laboratory counting the plates gave a higher count than the first laboratory, and in 71 plates the first laboratory reported the higher numbers. The amount of variation that would be found in duplicate counts of the same plates can be seen by examining Table IV, and it will be noticed that in some cases it was as great as 100 per cent, although as a rule the differences were less than this.

### A FIVE-DAY COUNT.

In order to determine whether any considerable increase in bacteria would be found by leaving the plates in incubation for five days instead of two, each of these plates, after being counted at the end of 48 hours, was set aside in the laboratory at room temperature for three days, when a second count was made. The results showed that the five-day count gave in most cases a slightly higher number of bacteria than the two-day count. This was not, however, uniform, and in some cases the five-day count was not as high as the two-day count. This was probably to be accounted for by the fact that some small specks were counted at the end of two days which were not real colonies, and this was discovered at the end of the five-day period. In some cases, however, the smaller five-day count was due to the fact that small colonies were overgrown by the increase in size of the larger colonies, and therefore were not seen at the end of the five days. The difference between the two-day count and the five-day count was not great enough to make any difference in the grading of any milk sample, and in general was less than the differences that could be seen in duplicate counts of the same plates made by two different laboratories on the same day.

### CREAM.

Among the lot of specimens submitted in this case there was one specimen of cream, and this gave results in the different laboratories far less uniform than those obtained in milk No. 74. This was of course to be expected, since there would be a less uniform distribution of bacteria in the sample. The analyses of laboratories A and B were, however, as close together as their analyses of milk, although they gave lower counts on this sample of cream than did the other two laboratories.

It was noticeable that the cream analyses made by inoculating the plates and subsequently pouring the agar into the tubes were much less uniform than those which were made when the diluted cream was put into the melted tube, rolled, and subsequently poured into the petri dish. This fact is doubtless explained by the greater difficulty of uniformly distributing the bacteria in cream by simply pouring the melted agar upon the diluted sample.

### SHAKING WITH BEADS.

In submitting samples No. 77 one of the three samples was taken from a bottle of milk after a simple agitation, and then a considerable number of sterile beads were placed in the bottle and the whole was subjected to an extremely violent shaking. After this two other samples were removed and sent to each of the laboratories. The three samples of 77 were therefore of the same milk, but one of them had been subjected to simple agitation and the others to a vigorous shaking with beads. The figures show that the vigorous shaking with the beads made practically no difference in the results. In each case the first sample of the three was the one that was submitted without the shaking with beads, and it can be seen that the figures here are no smaller than those obtained in the other two samples.

## THIRD SERIES OF TESTS.

The third series of tests was for the purpose of giving additional data concerning the limits of variation of the different laboratories, under conditions as nearly as possible identical, and also to test out several additional points in practical routine.

*Effect of shaking.*—The failure to obtain any difference in results in the test previously described, when the milk was shaken thoroughly with sterile beads, raised the question as to whether the shaking of the milk samples really made any noticeable difference in the final analysis. In the third test each laboratory was instructed to make one series of plates from each of the samples submitted, using simply a gentle agitation so as to mix the milk without shaking it, and to make a second series after giving it a normal vigorous shaking. The two plates were to be made at once under identical conditions in all other respects.

*The use of domestic peptone in comparison with that of Witte.*—The difficulty of obtaining Witte's peptone at the present time has led to an extension of the use of domestic peptone. The Digestive Ferment Co., of Detroit, submitted to the laboratories samples of four different lots of peptone made at different times, and two of these lots were distributed to the four laboratories for this test. Separate media were made both with the Witte and with each sample of the Detroit peptone and each of the milk samples submitted was tested thus by three media, identical in all respects except the peptone.

*Effect of icing.*—It is frequently necessary to keep a sample of milk for some hours before it can be submitted to laboratory analysis. To determine whether this can be done without materially affecting the results one series of tests was made as follows: From each of a series of milk bottles after thorough mixing there was removed one set of samples which was distributed to the test laboratories at 1 o'clock p. m. Immediately afterwards each bottle of mik was placed in a mixture of ice and water, the temperature of which was practically freezing, the result being that the milk was cooled to a temperature of slightly over 32° F. in a very short time. It was kept at this temperature until 9 o'clock the next morning. A second series of samples was then taken from each bottle and distributed to the different laboratories for a second series of plates. A comparison of the two results would show the general relation of the effect of keeping samples of milk for a period of 21 hours or thereabouts at a temperature of practically freezing.

*Direct microscopic examination of milk.*—In this series of tests there was also obtained a new set of data upon the bacterial examination of milk by means of the direct microscopic method. During the last few years a large amount of interest has developed concerning this method of bacteriological analysis of milk, which is calculated to give quicker results than the plate method; but no extended series of tests had been made to compare the accuracy of the bacteriological analysis by plate methods and by the microscopic method as modified in recent improvements. This series of experiments offered, therefore, an unusual opportunity for testing out the accuracy and availability of the direct microscopic method. Dr. R. S. Breed, of the Geneva Experiment Station, who has been more responsible than anyone else in perfecting and advocating the direct microscopic method, joined the other laboratories in this series of tests. Dr. Breed came to New York and made a series of smears of the milk samples submitted to laboratory A, while the various laboratories were making their plates. At the same time, the laboratory of the Board of Health made a similar series of smears from milk samples received by them. These smears were counted, and reports were sent to the referee without any comparison with the plate counts which had been obtained. The reports upon the bacteriological analysis by the microscopic method, therefore, were quite independent.

In reporting the results of the microscopic analyses in this series of tests there was a discrepancy between the method of counting adopted by the board of health laboratory and by Dr. Breed. The Board of Health laboratory reported the total number of individual bacteria that could be counted. Dr. Breed also gave a report of the total number of individual bacteria that could be found upon his smears; but in addition to this he gave another figure of much more significance in comparing the microscopic count with the plate count.

Every bacteriologist recognizes that a clump of bacteria in a milk sample will produce only one colony, whether it be made up of a single bacterium or of thousands. The bacteria in milk samples are more or less in clumps, and if the attempt is made to compare the results of the microscopic count with that of the plate count it is evident that the only significant figure in the microscopic count would be the number of groups or clumps that could be found, and not the number of individual bacteria. In the reports obtained from Dr. Breed there were given, in addition to the individual bacteria, the clumps which could be recognized by the direct microscopic examination.

In this series of tests, as in the last, each plate, after being counted, was passed along to a second laboratory, where a count was made. In the following table, for direct comparison, both counts are given, one immediately under the other, the first report being that of the laboratory making the plate and the second the count of the second laboratory to which the plates were submitted.

Three of the laboratories made a set of parallel tests with a medium which contained only one-half the quantity of peptone and of beef extract that is contained in the standard. The results with this medium were irregular and are given in the column headed "Dil. shake."

In the following table the columns headed "Det. A" and "Det. B" give the results obtained with the Detroit peptone as compared with the Witte peptone which was used in the standard given in the first column.

TABLE VI.

| Laboratory. | Plates 2 p. m. | | | After 21 hours icing. | | |
|---|---|---|---|---|---|---|
| | Stan. | Det. A. | Det. B. | S. Mix. | S. shake. | Dil. shake. |
| No. 78: | | | | | | |
| A rep | 1,650,000 | 2,010,000 | 1,880,000 | 1,500,000 | 5,300,000 | 3,450,000 |
| D rep | 1,800,000 | 2,640,000 | 1,970,000 | 1,950,000 | 4,840,000 | 1,300,000 |
| A rep | 1,540,000 | 1,470,000 | 880,000 | 1,550,000 | 2,840,000 | (?) |
| D rep | 1,690,000 | 2,530,000 | 2,100,000 | 2,610,000 | 5,470,000 | (?) |
| A rep | 470,000 | 350,000 | 490,000 | 1,890,000 | 7,200,000 | 3,650,000 |
| D rep | 510,000 | 290,000 | 460,000 | 2,830,000 | 4,880,000 | 2,210,000 |
| B rep | 860,000 | 457,000 | 660,000 | 1,800,000 | 4,900,000 | ............ |
| C rep | 600,000 | 362,000 | 590,000 | 1,600,000 | 2,750,000 | ............ |
| B rep | 260,000 | 77,000 | 1,960,000 | 3,340,000 | 6,400,000 | ............ |
| C rep | 750,000 | 100,000 | 1,310,000 | 2,350,000 | 4,000,000 | ............ |
| B rep | 1,250,000 | 1,180,000 | 1,400,000 | 2,050,000 | 1,560,000 | ............ |
| C rep | 900,000 | 1,360,000 | 1,920,000 | 1,820,000 | 4,500,000 | ............ |
| SHAKEN PLATES OF LABORATORY C NOT COUNTED BY LABORATORY B. | | | | | | |
| C rep | 625,000 | 600,000 | 660,000 | ............ | ............ | ............ |
| C rep | 1,220,000 | 1,310,000 | 1,200,000 | ............ | ............ | ............ |
| C rep | 2,400,000 | 2,300,000 | 2,300,000 | ............ | ............ | ............ |
| UNSHAKEN PLATES. | | | | | | |
| C rep | 600,000 | 800,000 | 540,000 | 2,100,000 | 5,700,000 | 5,300,000 |
| B rep | 530,000 | 700,000 | 620,000 | 4,800,000 | 5,600,000 | 5,600,000 |
| C rep | 570,000 | 600,000 | 540,000 | 2,250,000 | 8,750,000 | 6,000,000 |
| B rep | 750,000 | 810,000 | 680,000 | 2,160,000 | 10,700,000 | 7,600,000 |
| C rep | 580,000 | 1,100,000 | 770,000 | 4,000,000 | 4,750,000 | 4,250,000 |
| B rep | 480,000 | 1,160,000 | 840,000 | 4,500,000 | 6,240,000 | 4,250,000 |
| D rep | 800,000 | 1,520,000 | 1,100,000 | 970,000 | 910,000 | (?) |
| A rep | 450,000 | 860,000 | 880,000 | 970,000 | 910,000 | (?) |
| D rep | 460,000 | 600,000 | 900,000 | 1,200,000 | 67,000 | 45,000 |
| A rep | 470,000 | 910,000 | 900,000 | 1,220,000 | 1,040,000 | 35,000 |
| D rep | 430,000 | 400,000 | 380,000 | 1,540,000 | 5,300,000 | ............ |
| A rep | 260,000 | 339,000 | 270,000 | 1,050,000 | 4,100,000 | ............ |

## Table VI—Continued.

| Laboratory. | Plates 2 p. m. | | | After 21 hours icing. | | |
|---|---|---|---|---|---|---|
| | Stan. | Det. A. | Det. B. | S. Mix. | S. shake. | Dil. shake. |
| UNSHAKEN PLATES—contd. | | | | | | |
| No. 79: | | | | | | |
| A rep | 15,850,000 | 10,760,000 | 9,800,000 | 2,720,000 | 10,600,000 | 7,850,000 |
| D rep | 19,200,000 | 10,800,000 | 10,700,000 | 2,670,000 | 17,500,000 | 12,500,000 |
| A rep | 14,200,000 | 10,720,000 | 6,440,000 | 7,100,000 | 10,200,000 | 8,400,000 |
| D rep | 12,200,000 | 10,200,000 | 9,000,000 | 4,140,000 | 13,400,000 | 16,400,000 |
| B rep | 5,000,000 | 4,500,000 | 4,300,000 | 920,000 | 8,600,000 | ............ |
| C rep | 13,000,000 | 13,000,000 | 4,600,000 | 1,000,000 | 11,000,000 | ............ |
| B rep | 11,000,000 | 7,500,000 | 5,000,000 | 900,000 | 11,900,000 | ............ |
| C rep | 10,180,000 | 11,000,000 | 9,300,000 | 1,350,000 | 23,000,000 | ............ |
| THE SHAKEN PLATES NOT COUNTED BY LABORATORY B. | | | | | | |
| C rep | 28,000,000 | 3,000,000 | 2,500,000 | ............ | ............ | ............ |
| C rep | 8,200,000 | 15,300,000 | 9,600,000 | ............ | ............ | ............ |
| UNSHAKEN PLATES. | | | | | | |
| C rep | 6,320,000 | 6,000,000 | 7,400,000 | 4,850,000 | 24,000,000 | 29,000,000 |
| B rep | 5,200,000 | 5,000,000 | 5,800,000 | 1,430,000 | 32,000,000 | 35,000,000 |
| C rep | 3,710,000 | 3,240,000 | 2,900,000 | 5,600,000 | 19,000,000 | 18,000,000 |
| B rep | 4,200,000 | 2,260,000 | 3,080,000 | 6,600,000 | 10,800,000 | 6,400,000 |
| D rep | 1,700,000 | 2,610,000 | 1,300,000 | 830,000 | 3,270,000 | (?) |
| A rep | 4,760,000 | 2,470,000 | 1,760,000 | 2,360,000 | 4,400,000 | (?) |
| D rep | 2,800,000 | 3,000,000 | 2,900,000 | 920,000 | 3,500,000 | ............ |
| A rep | 1,940,000 | 2,760,000 | 2,660,000 | 740,000 | 2,280,000 | ............ |
| No. 80: | | | | | | |
| A rep | 58,000 | 59,000 | 63,000 | 30,000 | 67,000 | 83,000 |
| D rep | 75,000 | 73,000 | 70,000 | 40,000 | 89,000 | 74,000 |
| A rep | 89,000 | 79,000 | 54,000 | 34,000 | 57,000 | 36,000 |
| D rep | 108,000 | 109,000 | 100,000 | 71,000 | 48,000 | 40,000 |
| B rep | 61,000 | 75,000 | 70,000 | 40,000 | 69,000 | ............ |
| C rep | 68,000 | 65,000 | 61,000 | 46,000 | 61,000 | ............ |
| B rep | 60,000 | 70,000 | 47,000 | 44,000 | 63,000 | ............ |
| C rep | 55,000 | 103,000 | 49,000 | 45,000 | 65,000 | ............ |
| SHAKEN PLATES NOT COUNTED BY LABORATORY B. | | | | | | |
| C rep [1] | ............ | Missing | ............ | ............ | ............ | ............ |
| C rep | 132,000 | 129,000 | 121,000 | ............ | ............ | ............ |
| UNSHAKEN PLATES. | | | | | | |
| C rep | 92,000 | 72,000 | 79,000 | 53,000 | 120,000 | 105,000 |
| B rep | 91,000 | 83,000 | 83,000 | 55,000 | 148,000 | 168,000 |
| C rep | 60,000 | 57,000 | 50,000 | 65,000 | 127,000 | 135,000 |
| B rep | 62,000 | 61,000 | 58,000 | 74,000 | 260,000 | 224,000 |
| D rep | 62,000 | 72,000 | 67,000 | 28,000 | 30,000 | 9,000 |
| A rep | 40,000 | 44,000 | 40,000 | 33,000 | 22,000 | 8,000 |
| D rep | 62,000 | 66,000 | 65,000 | 25,000 | 17,000 | 12,000 |
| A rep | 48,000 | 50,000 | 32,000 | 21,000 | 17,000 | 22,000 |
| No. 81: | | | | | | |
| A rep | 350,000 | 386,000 | 198,000 | 250,000 | 470,000 | 300,000 |
| D rep | 2,440,000 | 400,000 | 270,000 | 390,000 | 500,000 | 350,000 |
| A rep | 354,000 | 520,000 | 190,000 | 190,000 | 375,000 | 150,000 |
| D rep | 343,000 | 390,000 | 170,000 | 226,000 | 318,000 | 225,000 |
| B rep | 160,000 | 92,000 | 84,000 | 98,000 | 143,000 | ............ |
| C rep | 165,000 | 120,000 | 90,000 | 90,000 | 161,000 | ............ |
| B rep | 116,000 | 100,000 | 112,000 | 104,000 | 95,000 | ............ |
| C rep | 160,000 | 125,000 | 80,000 | 100,000 | 99,000 | ............ |
| SHAKEN PLATES NOT COUNTED BY LABORATORY B. | | | | | | |
| C rep | 350,000 | 300,000 | 660,000 | ............ | ............ | ............ |
| C rep | 560,000 | 545,000 | 580,000 | ............ | ............ | ............ |
| UNSHAKEN PLATES. | | | | | | |
| C rep | 302,000 | 586,000 | 472,000 | 418,000 | 630,000 | 529,000 |
| B rep | 306,000 | 520,000 | 530,000 | 518,000 | 813,000 | 485,000 |
| C rep | 310,000 | 332,000 | 365,000 | 286,000 | 530,000 | 433,000 |
| B rep | 540,000 | 285,000 | 410,000 | 424,000 | 680,000 | 641,000 |
| D rep | 110,000 | 130,000 | 116,000 | 55,000 | 85,000 | 22,000 |
| A rep | 85,000 | 177,000 | 145,000 | 46,000 | 62,000 | 90,000 |
| D rep | 110,000 | 168,000 | 130,000 | 61,000 | 40,000 | 28,000 |
| A rep | 96,000 | 124,000 | 117,000 | 52,000 | 38,000 | 31,000 |

TABLE VI—Continued.

| Laboratory. | Plates 2 p. m. | | | After 21 hours icing. | | |
|---|---|---|---|---|---|---|
| | Stan. | Det. A. | Det. B. | S. Mix. | S. shake. | Dil. shake. |
| UNSHAKEN PLATES—contd. | | | | | | |
| No. 82: | | | | | | |
| A rep | 4,900 | 3,600 | 3,000 | 6,600 | 5,100 | 4,500 |
| D rep | 8,400 | 4,400 | 4,300 | 5,500 | 12,000 | 5,600 |
| A rep | 6,100 | 3,900 | 2,300 | 3,400 | 3,700 | 3,900 |
| D rep | 6,500 | 30,400 | 5,700 | 4,200 | 4,600 | 5,200 |
| B rep | 4,500 | 5,200 | 3,600 | 3,400 | 11,000 | ........... |
| C rep | 4,200 | 4,500 | 3,100 | 3,400 | 8,000 | ........... |
| B rep | 15,200 | 5,200 | 4,000 | 2,800 | 2,300 | ........... |
| C rep | 4,300 | 6,300 | 3,900 | 3,400 | 3,400 | ........... |
| SHAKEN PLATES NOT COUNTED BY LABORATORY B. | | | | | | |
| No. 82: | | | | | | |
| C rep | 4,800 | 5,500 | 4,700 | ........... | ........... | ........... |
| C rep | 4,800 | 8,500 | 4,600 | ........... | ........... | ........... |
| UNSHAKEN PLATES. | | | | | | |
| No. 82: | | | | | | |
| C rep | 4,300 | 6,100 | 4,500 | 4,300 | 5,600 | 4,100 |
| B rep | 3,300 | 4,200 | 3,100 | 4,600 | 7,100 | 3,900 |
| C rep | 3,600 | 14,000 | 3,400 | 3,300 | 5,400 | 4,500 |
| B rep | 3,600 | 13,500 | 3,600 | 4,600 | 6,500 | 4,600 |
| D rep | 2,100 | 4,400 | 3,300 | 2,500 | 1,900 | 2,400 |
| A rep | 2,100 | 3,800 | 2,700 | 2,500 | 1,600 | 1,700 |
| D rep | 1,200 | 3,500 | 4,000 | 2,100 | 2,600 | 1,000 |
| A rep | 5,300 | 2,800 | 2,100 | 1,900 | 2,300 | 1,300 |
| No. 83: | | | | | | |
| A rep | 16,700 | 16,100 | 11,400 | 7,800 | 5,900 | 6,800 |
| D rep | 17,100 | 14,600 | 11,000 | 13,100 | 12,500 | 11,900 |
| A rep | 16,600 | 19,200 | 13,400 | 6,900 | 10,900 | 12,200 |
| D rep | 17,000 | 17,300 | 13,400 | 12,600 | 19,200 | 18,100 |
| B rep | 15,900 | 16,800 | 12,500 | 8,000 | 12,100 | ........... |
| C rep | 15,400 | 13,500 | 12,500 | 8,200 | 12,900 | ........... |
| B rep | 9,000 | 12,000 | 12,300 | 12,000 | 9,800 | ........... |
| C rep | 16,800 | 20,000 | 12,000 | 14,000 | 10,800 | ........... |
| SHAKEN PLATES NOT COUNTED BY NORTH. | | | | | | |
| No. 83: | | | | | | |
| C rep | 23,000 | 25,000 | 24,000 | ........... | ........... | ........... |
| C rep | 21,000 | 23,000 | 22,000 | ........... | ........... | ........... |
| UNSHAKEN PLATES. | | | | | | |
| No. 83: | | | | | | |
| C rep | 11,000 | 12,000 | 12,700 | 13,000 | 25,000 | 25,000 |
| B rep | 13,100 | 10,800 | 13,800 | 13,000 | 26,000 | 14,000 |
| C rep | 13,500 | 11,100 | 11,900 | 10,900 | 20,000 | 17,000 |
| B rep | 13,100 | 10,800 | 13,800 | 10,300 | 26,000 | 17,200 |
| D rep | 10,100 | 14,000 | 11,900 | 5,900 | 6,200 | 1,300 |
| A rep | 6,500 | 7,300 | 11,800 | 4,900 | 4,900 | 1,400 |
| D rep | 9,700 | 10,200 | 11,600 | 5,100 | 7,000 | ........... |
| A rep | 5,900 | 6,400 | 7,100 | 4,000 | 4,200 | ........... |
| No. 84: | | | | | | |
| A rep | 14,700 | 13,700 | 12,700 | 4,500 | 10,300 | 5,600 |
| D rep | 15,000 | 12,100 | 14,100 | 6,400 | 10,500 | 8,600 |
| A rep | 8,600 | 10,300 | 7,100 | 5,800 | 7,300 | 9,700 |
| D rep | 10,200 | 10,200 | 8,000 | 6,700 | 11,100 | 19,400 |
| B rep | 17,000 | 10,000 | 14,000 | 6,200 | 6,000 | ........... |
| C rep | 16,000 | 12,000 | 14,000 | 9,000 | 12,000 | ........... |
| B rep | 9,100 | 12,500 | 9,600 | 5,000 | 9,800 | ........... |
| C rep | 7,100 | 102,000 | 9,500 | 5,600 | 10,400 | ........... |
| SHAKEN PLATES NOT COUNTED BY LABORATORY B. | | | | | | |
| No. 84: | | | | | | |
| C rep | 15,600 | 14,400 | 13,800 | ........... | ........... | ........... |
| C rep | 16,500 | 15,900 | 14,900 | ........... | ........... | ........... |
| UNSHAKEN PLATES. | | | | | | |
| No. 84: | | | | | | |
| C rep | 7,500 | 8,000 | 13,200 | 8,400 | 16,000 | 15,800 |
| B rep | 14,000 | 8,600 | 15,300 | 9,600 | 18,400 | 9,200 |
| C rep | 6,800 | 6,300 | 6,000 | 22,000 | 26,000 | 16,500 |
| B rep | 6,400 | 7,000 | 6,200 | 19,800 | 24,000 | 7,600 |
| D rep | 6,000 | 9,600 | 8,500 | 2,000 | 5,900 | 3,500 |
| A rep | 8,900 | 7,400 | 5,600 | 1,900 | 4,000 | 700 |
| D rep | 5,500 | 8,200 | 7,500 | 3,400 | 5,300 | ........... |
| A rep | 4,300 | 5,200 | 5,300 | 2,600 | 4,700 | ........... |

## General Summary of Third Series.

### VARIATIONS IN RESULTS.

In this series the results are in about as close agreement as they were in the second series, as above reported. Although there are individual discrepancies and some variations in averages, in general they agree with each other fairly well. This is shown best in the tables, VII and VIII, which at the same time show the degree of accuracy that may be expected when different laboratories analyze samples of the same bottle of milk. In this table are given the general averages of the tests of the same bottle of milk and also the general average that each laboratory has obtained in the analysis of the samples submitted to it. In the first column are given the averages obtained with media made with the Witte peptone (marked Stan) and with the Detroit peptone of two lots (marked A and B).

TABLE VII.—*Summary of averages from table.*

| No. | Media. | Plate counts. | | | Microscopic group count. | |
|---|---|---|---|---|---|---|
| | | Shaken. | Unshaken. | After icing, shaken. | Before icing. | After icing. |
| 80 | Stan | 70,000 | 44,000 | 78,000 | 29,000 | 22,000 |
| | Det. A | 75,000 | | | | |
| | Det. B | 65,000 | | | | |
| 78 | Stan | 920,000 | 2,170,000 | 4,300,000 | 1,170,000 | 3,400,000 |
| | Det. A | 1,000,000 | | | | |
| | Det. B | 1,100,000 | | | | |
| 81 | Stan | 217,000 | 151,000 | 260,000 | 245,000 | 219,000 |
| | Det. A | 250,000 | | | | |
| | Det. B | 210,000 | | | | |
| 82 | Stan | 4,200 | 3,600 | 4,500 | 1,700 | 10,000 |
| | Det. A | 10,900 | | | | |
| | Det. B | 3,200 | | | | |
| 83 | Stan | 12,600 | 9,300 | 12,500 | 7,500 | 12,700 |
| | Det. A | 15,400 | | | | |
| | Det. B | 14,500 | | | | |
| 84 | Stan | 11,000 | 7,400 | 11,400 | 4,500 | 7,700 |
| | Det. A | 11,700 | | | | |
| | Det. B | 10,300 | | | | |
| 79 | Stan | 10,600,000 | 2,800,000 | 11,400,000 | 10,000,000 | 2,770,000 |
| | Det. A | 9,000,000 | | | | |
| | Det. B | 6,600,000 | | | | |

The following summary gives the general average of all the analyses of each sample of milk and also the average obtained by each of the laboratories. It will serve, therefore, to show how close to the general average were the results of the individual laboratories.

TABLE VIII.—*Averages of shaken plates.*

| Number. | General average. | Laboratory A. | Laboratory B. | Laboratory C. | Laboratory D. |
|---|---|---|---|---|---|
| 80 | 70,000 | 82,000 | 61,000 | 132,000 | 53,000 |
| 78 | 920,000 | 1,300,000 | 770,000 | 1,400,000 | 480,000 |
| 81 | 217,000 | 338,000 | 150,000 | 450,000 | 98,000 |
| 82 | 4,200 | 6,500 | 7,000 | 4,800 | 2,700 |
| 83 | 12,600 | 16,800 | 14,300 | 22,500 | 8,000 |
| 84 | 11,000 | 12,100 | 12,300 | 16,000 | 6,100 |
| 79 | 10,000,000 | 15,400,000 | 10,500,000 | 18,000,000 | 2,770,000 |

### COMPARISON OF THE RESULTS OF DIFFERENT LABORATORIES.

In these tables is seen far less divergence in the results than in the first series of experiments, but at the same time it will be noticed that there is still a somewhat general relation of the laboratories to each other. It will be seen from the summary table just given that laboratory D in every case gave a noticeably lower count than either of the other three laboratories. No one of the other three, however, gave uniformly higher results, as in the first series of tests. The actual figures were as follows:

Laboratory C, in 77 per cent of its tests, was above the general average; laboratory A in 70 per cent was above the general average; laboratory B in 51 per cent was above the general average, while laboratory D was above the general average in only 5 per cent. On the other hand, laboratory D was below the general average in 95 per cent of its counts, while laboratory C was below the average in only 23 per cent of its counts.

*Range of variation.*—From the last table it will be seen that the range of variation in the laboratories is considerable, even under the most uniform conditions and when averages are used. There is a variation in some cases of 300 per cent between the lowest average and the highest average analyses of these samples of milk from the same bottle.

*Variation in samples.*—One of the most significant conclusions of this series is that samples obtained from the same bottle of milk clearly vary noticeably in the number of bacteria which they contain. For example, No. 78, Table VI, represents three different samples from the same bottle of milk, distributed to each of the four laboratories. The bottle of milk containing these samples was first thoroughly mixed by pouring it back and forth from one bottle to another several times, so as to produce as thorough a mixture as possible, without, however, any particular shaking. The samples were then taken one after the other by means of a sterile pipette. By examining Table VI it will be seen that the three samples received by laboratory A gave analyses in round numbers, 1,600,000, 1,500,000, and 400,000. The sample that gave 400,000 gave practically the same number in all of the three media that were used in this test, and also gave essentially the same results when the plates were counted by both laboratories A and D, thus showing that there was an actual difference in the number of colonies on the plate, and not simply a difference in the method of counting. This shows that there was an actual difference in the number of bacteria in the sample, and that the difference in analysis was not due simply to laboratory technique. It is also interesting to note that the microscopic examination made by Breed of this same sample of milk in laboratory A gave a smaller bacterial count of groups in this sample than it did in the other two samples of the same bottle of milk. These facts together show beyond question that two samples of milk from the same bottle, even after thorough mixing, may vary quite noticeably in their bacterial content.

*Variations in duplicate counts.*—A comparison of the figures of duplicate counts of the same plates is interesting as showing again that even when great care is used two laboratories will not in all cases obtain counts agreeing with each other very closely. In a few cases the differences were extreme, due to a failure of one laboratory to recognize small specks as colonies, or to the second laboratory's counting dirt specks as colonies. But aside from these differences there is a general variation in the duplicate counts of the same plates which ranges from 100 per cent downward, although it is rarely over 50 per cent. From this we must conclude that an allowance of at least 50 per cent of error must be made for the personal factor in counting the plates.

*Domestic peptone compared to Witte's peptone.*—The results of these plates show that the two samples of domestic peptone gave practically the same results as those of Witte. In this series the averages of the three media were very close to each other, the average obtained by the Witte being actually between the average obtained from the two Detroit peptones. The differences were so slight, however, as to indicate no advantage of the one peptone over the other.

*Icing.*—These tables show that there is a very slight increase in bacteria in 21 hours when the milk samples are placed in the mixture of melting ice and water. In the freshest samples of milk, where the bacteria were few in the original sample, the increase during the 21 hours is so slight as to be negligible. Sometimes there is no increase at all, and in one or two cases there has been an apparent decrease. In other samples, where the bacteria were in millions at the outset, there was a slight increase during the 21 hours. In one case the increase was fourfold. In all others it was less than this, and in most cases it was less than 20 per cent. This fact shows that it is possible to preserve samples from analysis for 20 hours without very great change in bacterial content, providing they be immersed in melting ice and water.

*Effect of shaking.*—The effect of shaking was evident, but it was not very great. In three cases there was an increase of two or three fold in bacterial count as the result of shaking. In all others it was less than this. The smallest increase brought about by shaking was 30 per cent.

## RESULTS OF THE DIRECT MICROSCOPIC EXAMINATION.

The most interesting conclusion in this series of tests was the comparison of the plate counts with the direct microscopic count. The summary table following is prepared, giving these results in such a way as to make them most easily comparable with each other.

TABLE IX.—*Direct microscopic counts compared with the average plate counts.*

| No. | Count. | Before icing. | | | After icing. | | |
|---|---|---|---|---|---|---|---|
| | | Average plate count. | Microscopic groups. | Individuals. | Average plate count. | Microscopic groups. | Individuals. |
| 82 | By Br | 6,000 | 1,500 | 3,000 | 4,500 | 5,800 | 16,000 |
| | By Bd | 6,000 | ............ | 50,000 | 4,500 | ............ | 900,000 |
| | By Br | 6,000 | 2,000 | 6,500 | 4,500 | 1,500 | 8,000 |
| | By Bd | 6,000 | ............ | 65,000 | 4,500 | ............ | 425,000 |
| 83 | By Br | 12,600 | 6,000 | 67,000 | 12,500 | 10,500 | 56,000 |
| | By Bd | 12,600 | ............ | 131,000 | 12,500 | ............ | 67,000 |
| | By Br | 12,600 | 3,000 | 29,000 | 12,500 | 5,000 | 44,000 |
| | By Bd | 12,600 | ............ | [1]16,000 | 12,500 | ............ | 1,700,000 |
| 84 | By Br | 11,000 | 7,000 | 97,000 | 11,400 | 8,000 | 52,000 |
| | By Bd | 11,000 | ............ | 32,000 | 11,400 | ............ | [2]65,000 |
| | By Br | 11,000 | 2,500 | 29,000 | 11,400 | 4,000 | 24,500 |
| | By Bd | 11,000 | ............ | [3]90,000 | 11,400 | ............ | [4]210,000 |
| 80 | By Br | 70,000 | 24,500 | 67,000 | 78,000 | 15,000 | 208,000 |
| | By Bd | 70,000 | ............ | 150,000 | 78,000 | ............ | 131,000 |
| | By Br | 70,000 | 34,950 | 333,000 | 78,000 | 28,000 | 267,000 |
| | By Bd | 70,000 | ............ | 390,000 | 78,000 | ............ | 1,000,000 |
| 81 | By Br | 217,000 | 245,000 | 948,000 | 260,000 | 212,000 | 2,130,000 |
| | By Bd | 217,000 | ............ | 2,500,000 | 260,000 | ............ | 2,900,000 |
| | By Br | 217,000 | 128,000 | 592,000 | 260,000 | 226,000 | 991,000 |
| | By Bd | 217,000 | ............ | 3,100,000 | 260,000 | ............ | 2,000,000 |
| 78 | By Br | 920,000 | 1,900,000 | 7,968,000 | 4,300,000 | 2,230,000 | 15,000,000 |
| | By Bd | 920,000 | ............ | 23,000,000 | 4,300,000 | ............ | 21,000,000 |
| | By Br | 920,000 | 981,000 | 3,500,000 | 4,300,000 | 3,500,000 | 16,000,000 |
| | By Bd | 920,000 | ............ | 70,000,000 | 4,300,000 | ............ | 8,000,000 |
| | By Br | 920,000 | 557,000 | 22,900,000 | 4,300,000 | 3,420,000 | 20,000,000 |
| | By Bd | 920,000 | ............ | 24,000,000 | 4,300,000 | ............ | 3,000,000 |
| 79 | By Br | 10,000,000 | 13,617,000 | 240,000,000 | 14,000,000 | 2,563,000 | 131,000,000 |
| | By Bd | 10,000,000 | ............ | 150,000,000 | 14,000,000 | ............ | 460,000,000 |
| | By Br | 10,000,000 | 6,800,000 | 95,000,000 | 14,000,000 | 2,982,000 | 145,000,000 |
| | By Bd | 10,000,000 | ............ | 243,000,000 | 14,000,000 | ............ | 90,000,000 |

[1] Less than 16,000. [2] Less than 65,000. [3] Less than 90,000. [4] Less than 210,000.

In the above table Br. refers to the microscopic counts by Breed in Borden's laboratory, and Bd. to the counts made by the Board of Health.

Two samples of each bottle of milk were examined by each laboratory and three samples of No. 78. The microscopic count of each sample is given above, but the plate count average is the average of all the plate counts made from the same bottle, this general average being given as most representative:

From this table several conclusions can be drawn:

1. The direct microscopic count classifies samples of milk into grades A, B, and C as accurately as will the plate count of the same samples. When groups of bacteria are counted instead of individuals, these samples of milk will agree in grade, whether they were graded by the plate method or the direct microscopic method.

2. It is evident from these tests that the microscope alone can give an actual indication of the number of bacteria in the milk. The plate method is obviously inadequate to give anything but groups. Where the milk is filled with a type of bacteria that has a tendency to cling in clumps, the plate method alone can give no adequate knowledge of the number of bacteria present.

3. The rather close agreement of the plate count with the group count obtained by Breed upon the same samples of milk shows that

in these samples there was no noticeable counting of dead bacteria, indicating that in fresh milk which has been subjected to normal conditions practically all of the bacteria are alive, at least for a period of 24 hours from the time of milking.

4. Criticism has been made against the Breed method of microscopic examination on the ground that the use of 1/100 c. c. is inaccurate, since such a small amount can not be regarded as giving a fair sample of the milk. It has been claimed that the plate method, which starts with a whole cubic centimeter, will give more accurate results in this respect, even though this is subsequently diluted with water. A comparison of the microscopic group counts in the above tables with the plate counts of the same samples is a complete answer to this criticism.

5. A second criticism of the microscopic method is that it is applicable only to samples of milk containing large numbers of bacteria and not practical in milk where the bacteria are only a few thousand per c. c. The reason for this criticism is that the microscopic field covers such a small portion of a cubic centimeter of milk that the factor by which the number of bacteria found per field must be multiplied is so large as to make it unusable where the number of bacteria is small. The figures in the above table are also a complete answer to this criticism, for the counts of Breed upon the low counting samples are equally accurate with the higher counting samples and agree quite satisfactorily with the plate counts upon the same samples of milk.

Inasmuch as the laboratory of the Board of Health did not in this case count groups, but only individuals, no comparison can be made between their counts and those of the plates. In general magnitude of the number of individuals per c. c., however, it will be seen that the microscopic count of the board of health agrees fairly well with the individual count of Breed.

## FOURTH SERIES OF TESTS.

The extremely interesting results obtained by a comparison of the microscopic tests with the plate counts in the third series of tests led to the planning of a fourth series, in which the microscope should be used more extensively for comparison. In this case Dr. Breed came to New York, and brought with him Dr. Brew, who has been working with him in the Geneva Experiment Station upon the microscopic method. Dr. Breed made smears of the samples of milk received by the Board of Health laboratory and Dr. Brew made smears of milk submitted to the laboratory of Borden. The Board of Health and the Borden laboratories also made smears from the same samples, and the reports were submitted to the referee without comparison with each other. In this last series it was also to be understood that groups should be counted rather than individuals. Dr. Breed and Dr. Brew both made individual counts also, but the Board of Health and Borden laboratories made only group counts. The Board of Health laboratory, in addition to using a 1/100 c. c. pipette, used also the platinum loop method of obtaining the 1/100 c. c. of milk for smearing.

In this last series a further test was made of two more lots of peptone from the Digestive Ferment Co., as compared with the media made up from Witte's peptone.

In this series each laboratory counted its plates at the end of 24 hours and again at the end of 48 hours, for the purpose of finding whether the 24-hour count is manifestly inferior to the 48-hour count. It was to be expected that the 24-hour count would be somewhat less; but the advantage of saving 24 hours is obvious to anyone who has had anything to do with the technique of milk inspection, and it has been strongly urged that a 24-hour count might be advantageously substituted for a 48-hour count, which is now required by standard methods.

In this series of tests a slight difference in the reporting of the tests was made. In the previous series the figures given as the final report and published in the previous tables were those determined by the referee in studying the different counts of the different dilutions as submitted to him. In this last series, however, each laboratory was requested to make its own final report, and the figures reported are the reports that the laboratories themselves would send out as the results of their analyses of their own plates. There is, however, practically no difference between the results here reported and those that have hitherto been reported by the referee, except that in a few cases in the previous tables the final report was obtained by averaging the reports of two different dilutions, whereas in these cases the reports were, as a rule, obtained from a single report made, which gave the best number of colonies per plate. In the following table the count of the second laboratory is placed immediately under that of the first, to make easy comparison possible. The first four columns are plate counts and the last two microscopic counts. The third column was obtained by the use of dilute media like that in the last series of tests.

TABLE X.

| | Stan. | Det. | Dilute. | 24 hours. | Microscopic groups. | Microscopic individuals. |
|---|---|---|---|---|---|---|
| No. 85: | | | | | | |
| A rep | 81,000,000 | 38,000,000 | 72,000,000 | 19,800,000 | Bo. 15,500,000 | ............ |
| B rep | 27,000,000 | 38,000,000 | 31,000,000 | ............ | Br. 54,520,000 | 231,000,000 |
| A rep | 34,000,000 | 61,000,000 | 38,000,000 | 42,000,000 | Bo. 5,700,000 | ............ |
| B rep | 25,000,000 | 22,000,000 | 35,000,000 | ............ | Br. 40,000,000 | 181,000,000 |
| A rep | 79,000,000 | 48,000,000 | 81,000,000 | 54,000,000 | Bo. 9,000,000 | ............ |
| B rep | 20,000,000 | 29,000,000 | 28,000,000 | ............ | Br. 36,000,000 | 149,000,000 |
| B rep | 31,000,000 | 26,000,000 | 22,000,000 | 33,320,000 | ............ | ............ |

Table X—Continued.

| | Stan. | Det. | Dilute. | 24 hours. | Microscopic groups. | Microscopic individuals. |
|---|---|---|---|---|---|---|
| No. 85—Continued. | | | | | | |
| A rep | 56,000,000 | 15,000,000 | 16,000,000 | | | |
| B rep | 40,000,000 | 37,000,000 | 31,000,000 | 4,000,000 | | |
| A rep | 34,200,000 | 61,000,000 | 38,000,000 | | | |
| B rep | 34,000,000 | 31,000,000 | 36,000,000 | 2,900,000 | | |
| A rep | 79,000,000 | 48,000,000 | 81,000,000 | | | |
| C rep | 27,000,000 | 37,000,000 | 38,000,000 | 34,000,000 | Bd. of H. 38,000,000 | |
| D rep | 36,000,000 | 36,000,000 | 40,000,000 | | Breed 50,000,000 | Group. |
| C rep | 37,000,000 | 41,000,000 | 23,000,000 | 44,000,000 | Bd. of H. 45,500,000 | |
| D rep | 40,000,000 | 36,000,000 | 37,000,000 | | Breed 45,000,000 | Group. |
| C rep | 24,000,000 | 30,000,000 | 34,000,000 | 48,000,000 | Bd. of H. 42,000,000 | |
| D rep | 23,000,000 | 21,000,000 | 40,000,000 | | Breed 43,000,000 | Group. |
| D rep | 2,600,000 | 9,200,000 | 31,000,000 | 1,300,000 | | |
| C rep | 2,040,000 | 10,000,000 | 3,210,000 | | | |
| D rep | 10,000,000 | 1,500,000 | 13,000,000 | 600,000 | | |
| C rep | 1,970,000 | 1,890,000 | 16,200,000 | | | |
| D rep | 15,000,000 | 15,000,000 | 40,000,000 | 18,000,000 | | |
| C rep | 13,600,000 | 14,500,000 | 13,200,000 | | | |
| No. 86: | | | | | | |
| A rep | 410,000 | 231,000 | 420,000 | 280,000 | Bo. 89,000 | |
| B rep | 390,000 | 320,000 | 400,000 | | Br. 147,000 | 4,428,000 |
| A rep | 279,000 | 266,000 | 258,000 | 270,000 | Bo. 76,000 | |
| B rep | 247,000 | 210,000 | 185,000 | | Br. 157,000 | 3,933,000 |
| A rep | 320,000 | 500,000 | 315,000 | 324,000 | Bo. 185,000 | |
| B rep | 550,000 | 235,000 | 306,000 | | Br. 123,000 | 3,278,000 |
| B rep | 392,000 | 264,000 | 231,000 | 228,000 | | |
| A rep | 144,000 | 228,000 | 156,000 | | | |
| B rep | 245,000 | 240,000 | 141,000 | 170,000 | | |
| A rep | 132,000 | 237,000 | 63,000 | | | |
| B rep | 326,000 | 240,000 | 225,000 | 255,000 | | |
| A rep | 220,000 | 260,000 | 142,000 | | | |
| C rep | 400,000 | 310,000 | 287,000 | 245,000 | Bd. of H. 484,000 | |
| D rep | 360,000 | 330,000 | 340,000 | | Breed 224,000 | Group. |
| C rep | 310,000 | 260,000 | 268,000 | 230,000 | Bd. of H. 476,500 | |
| D rep | 350,000 | 360,000 | 400,000 | | Breed 267,000 | Group. |
| C rep | 232,000 | 213,000 | 255,000 | 250,000 | Bd. of H. 558,000 | |
| D rep | 300,000 | 250,000 | 300,000 | | Breed 220,000 | Group. |
| D rep | 83,000 | 110,000 | 90,000 | 67,000 | | |
| C rep | 83,000 | 124,000 | 89,000 | | | |
| D rep | 130,000 | 150,000 | 140,000 | 130,000 | | |
| C rep | 118,000 | 136,000 | 143,000 | | | |
| D rep | 140,000 | 160,000 | 150,000 | 140,000 | | |
| C rep | 129,000 | 116,000 | 119,000 | | | |
| No. 87: | | | | | | |
| A rep | 550,000 | 352,000 | 480,000 | 432,000 | Bo. (—) | |
| B rep | 380,000 | 428,000 | 600,000 | | Br. 185,000 | 1,095,000 |
| A rep | 505,000 | 299,000 | 580,000 | 538,000 | Bo. 360,000 | |
| B rep | 800,000 | 440,000 | 710,000 | | Br. 190,000 | 558,000 |
| A rep | 600,000 | 660,000 | 545,000 | 544,000 | Bo. 270,000 | |
| B rep | 400,000 | 540,000 | 780,000 | | Br. 186,000 | 795,000 |
| B rep | 600,000 | 570,000 | 710,000 | 600,000 | | |
| A rep | 540,000 | 326,000 | 470,000 | | | |
| B rep | 560,000 | 290,000 | 288,000 | 375,000 | | |
| A rep | 700,000 | 146,000 | 115,000 | | | |
| B rep | 348,000 | 700,000 | 516,000 | 430,000 | | |
| A rep | 316,000 | 310,000 | 555,000 | | | |
| C rep | 208,000 | 212,000 | 78,000 | 155,000 | Bd. of H. 480,000 | |
| D rep | 230,000 | 240,000 | 120,000 | | Breed 268,000 | |
| C rep | 311,000 | 274,000 | 182,000 | 275,000 | Bd. of H. 625,000 | |
| D rep | 300,000 | 280,000 | 200,000 | | Breed 275,000 | |
| C rep | 470,000 | 580,000 | 400,000 | 480,000 | Bd. of H. 925,000 | |
| D rep | 480,000 | 550,000 | 390,000 | | Breed 405,000 | |
| D rep | 120,000 | 72,000 | 87,000 | 71,000 | | |
| C rep | 152,000 | 64,000 | 81,000 | | | |
| D rep | 120,000 | 100,000 | 78,000 | 87,000 | | |
| C rep | 120,000 | 95,000 | 120,000 | | | |
| D rep | 290,000 | 230,000 | 210,000 | 220,000 | | |
| C rep | 219,000 | 184,000 | 259,000 | | | |
| No. 88: | | | | | | |
| A rep | 496,000 | 520,000 | 410,000 | 336,000 | Bo. 300,000 | |
| B rep | 480,000 | 392,000 | 390,000 | | Br. 121,000 | 904,000 |
| A rep | 188,000 | 320,000 | 170,000 | 243,000 | Bo. 252,000 | |
| B rep | 186,000 | 340,000 | 390,000 | | Br. 104,000 | 1,018,000 |
| A rep | 298,000 | 253,000 | 232,000 | 293,000 | Bo. 80,000 | |
| B rep | 121,000 | 262,000 | 240,000 | | Br. 66,000 | 959,000 |
| B rep | 680,000 | 412,000 | 230,000 | 490,000 | | |
| A rep | 600,000 | 322,000 | 151,000 | | | |
| B rep | 640,000 | 480,000 | 460,000 | 486,000 | | |
| A rep | 580,000 | 460,000 | 390,000 | | | |
| B rep | 624,000 | 400,000 | 145,000 | 517,000 | | |
| A rep | 500,000 | 244,000 | 116,000 | | | |

### Table X—Continued.

| | Stan. | Det. | Dilute. | 24 hours. | Microscopic groups. | Microscopic individuals. |
|---|---|---|---|---|---|---|
| No. 88—Continued. | | | | | | |
| C rep | 294,000 | 292,000 | 124,000 | 256,000 | Bd. of H. 336,000 | ...... |
| D rep | 280,000 | 143,000 | 284,000 | ...... | Breed 147,000 | Group |
| C rep | 159,000 | 250,000 | 244,000 | 110,000 | Bd. of H. 345,000 | ...... |
| D rep | 156,000 | 151,000 | 250,000 | ...... | Breed 74,000 | Group. |
| C rep | 248,000 | 167,000 | 156,000 | 142,000 | Bd. of H. 242,000 | ...... |
| D rep | 239,000 | 147,000 | 171,000 | ...... | Breed 107,000 | Group. |
| D rep | 230,000 | 140,000 | 110,000 | 110,000 | ...... | ...... |
| C rep | 145,000 | 208,000 | 122,000 | ...... | ...... | ...... |
| D rep | 200,000 | 200,000 | 160,000 | 100,000 | ...... | ...... |
| C rep | 260,000 | 216,000 | 148,000 | ...... | ...... | ...... |
| D rep | 190,000 | 120,000 | 90,000 | 82,000 | ...... | ...... |
| C rep | 100,000 | 95,000 | 80,000 | ...... | ...... | ...... |
| No. 89: | | | | | | |
| A rep | 289,000 | 274,000 | 301,000 | 288,000 | Bo. 319,000 | ...... |
| B rep | 310,000 | 360,000 | 420,000 | ...... | Br. 321,000 | 862,000 |
| A rep | 514,000 | 258,000 | 540,000 | 264,000 | Bo. 175,000 | ...... |
| B rep | 480,000 | 720,000 | 416,000 | ...... | Br. 345,000 | 1,075,000 |
| A rep | 179,000 | 173,000 | 260,000 | 141,000 | Bo. 216,000 | ...... |
| B rep | 308,000 | 284,000 | 304,000 | ...... | Br. 237,000 | 693,000 |
| B rep | 88,000 | 95,000 | 260,000 | 43,000 | ...... | ...... |
| A rep | 97,000 | 95,000 | 66,000 | ...... | ...... | ...... |
| B rep | 104,000 | 96,000 | 138,000 | 90,000 | ...... | ...... |
| A rep | 104,000 | 121,000 | 118,000 | ...... | ...... | ...... |
| B rep | 400,000 | 168,000 | 156,000 | 260,000 | ...... | ...... |
| A rep | 366,000 | 310,000 | 127,000 | ...... | ...... | ...... |
| C rep | 506,000 | 370,000 | 172,000 | 286,000 | Bd. of H. 975,000 | ...... |
| D rep | 525,000 | 182,000 | 430,000 | ...... | Breed 593,000 | Group. |
| C rep | 530,000 | 440,000 | 316,000 | 400,000 | Bd. of H. 468,000 | ...... |
| D rep | 500,000 | 334,000 | 470,000 | ...... | Breed 452,000 | Group. |
| C rep | 430,000 | 465,000 | 236,000 | 315,000 | Bd. of H. 493,000 | ...... |
| D rep | 513,000 | 146,000 | 600,000 | ...... | Breed 450,000 | Group. |
| D rep | 360,000 | 350,000 | 130,000 | 320,000 | ...... | ...... |
| C rep | 300,000 | 183,000 | 135,000 | ...... | ...... | ...... |
| D rep | 340,000 | 320,000 | 20,000 | 100,000 | ...... | ...... |
| C rep | 300,000 | 340,000 | 30,000 | ...... | ...... | ...... |
| D rep | 320,000 | 270,000 | 70,000 | 13,000 | ...... | ...... |
| C rep | 285,000 | 274,000 | 43,000 | ...... | ...... | ...... |
| No. 90: | | | | | | |
| A rep | 145,000 | 130,000 | 166,000 | 128,000 | Bo. (—) | ...... |
| B rep | 152,000 | 132,000 | 137,000 | ...... | Br. 30,000 | 294,000 |
| A rep | 206,000 | 182,000 | 138,000 | 88,000 | Bo. 115,000 | ...... |
| B rep | 159,000 | 146,000 | 168,000 | ...... | Br. 23,000 | 241,000 |
| B rep | 184,000 | 165,000 | 126,000 | 106,000 | ...... | ...... |
| A rep | 76,000 | 166,000 | 88,000 | ...... | ...... | ...... |
| B rep | 160,000 | 166,000 | 131,000 | 140,000 | ...... | ...... |
| A rep | 144,000 | 99,000 | 106,000 | ...... | ...... | ...... |
| C rep | 120,000 | 210,000 | 143,000 | 232,000 | Bd. of H. 98,000 | ...... |
| D rep | 140,000 | 300,000 | 200,000 | ...... | Breed 63,000 | Group. |
| C rep | 208,000 | 85,000 | 142,000 | 105,000 | Bd. of H. 140,000 | ...... |
| D rep | 200,000 | 200,000 | 170,000 | ...... | Breed 67,000 | Group. |
| D rep | 63,000 | 44,000 | 42,000 | 47,000 | ...... | ...... |
| C rep | 53,000 | 44,000 | 46,000 | ...... | ...... | ...... |
| D rep | 70,000 | 48,000 | 52,000 | 56,000 | ...... | ...... |
| C rep | 61,000 | 51,000 | 60,000 | ...... | ...... | ...... |
| No. 91: | | | | | | |
| A rep | 2,180,000 | 560,000 | 1,260,000 | 234,000 | Bo. 310,000 | ...... |
| B rep | 1,730,000 | 530,000 | 1,460,000 | ...... | Br. 368,000 | 2,150,000 |
| A rep | 1,310,000 | 1,110,000 | 1,810,000 | 680,000 | Bo. 676,000 | ...... |
| B rep | 1,240,000 | 1,250,000 | 1,270,000 | ...... | Br. 535,000 | 3,347,000 |
| A rep | 1,950,000 | 1,360,000 | 1,060,000 | 430,000 | ...... | ...... |
| B rep | 1,600,000 | 1,250,000 | 980,000 | ...... | ...... | ...... |
| B rep | 1,740,000 | 1,400,000 | 1,650,000 | 460,000 | ...... | ...... |
| A rep | 1,380,000 | 990,000 | 1,110,000 | ...... | ...... | ...... |
| B rep | 2,240,000 | 1,340,000 | 2,500,000 | 460,000 | ...... | ...... |
| A rep | 2,010,000 | 780,000 | 2,100,000 | ...... | ...... | ...... |
| B rep | 1,640,000 | 1,500,000 | 1,330,000 | 322,000 | ...... | ...... |
| A rep | 1,460,000 | 960,000 | 1,010,000 | ...... | ...... | ...... |
| C rep | 1,190,000 | 1,390,000 | 1,370,000 | 1,800,000 | Bd. of H. 907,000 | ...... |
| D rep | 1,200,000 | 1,700,000 | 1,200,000 | ...... | Breed 485,000 | ...... |
| C rep | 890,000 | 1,280,000 | 1,410,000 | 1,250,000 | Bd. of H. 1,224,000 | ...... |
| D rep | 900,000 | 1,300,000 | 1,300,000 | ...... | Breed 558,000 | ...... |
| C rep | 1,650,000 | 2,200,000 | 1,190,000 | 1,400,000 | Bd. of H. 8,832,000 | ...... |
| D rep | 1,600,000 | 2,700,000 | 1,150,000 | ...... | Breed 895,000 | ...... |
| D rep | 460,000 | 500,000 | 430,000 | 410,000 | ...... | ...... |
| C rep | 535,000 | 573,000 | 420,000 | ...... | ...... | ...... |
| D rep | 370,000 | 350,000 | 350,000 | 220,000 | ...... | ...... |
| C rep | 500,000 | 560,000 | 400,000 | ...... | ...... | ...... |
| D rep | 356,000 | 260,000 | 350,000 | 250,000 | ...... | ...... |
| C rep | 430,000 | 216,000 | 158,000 | ...... | ...... | ...... |

TABLE XI.—*Microscopic count, showing comparison of the group and the individual count, and also the loop as compared with the pipette method.*

| No. | Gr. Groups. | | Individuals. | Pipette method. | Loop method. |
|---|---|---|---|---|---|
| 85 | By Brew........ | 54,500,000 | 231,000,000 | ........ | ........ |
| | | 40,000,000 | 181,000,000 | ........ | ........ |
| | | 36,000,000 | 149,000,000 | ........ | ........ |
| | Breed........ | 50,000,000 | 365,000,000 | Bd. of H. 37,000,000 | 39,000,000 |
| | | 45,000,000 | 237,000,000 | 42,800,000 | 49,000,000 |
| | | 43,000,000 | 203,000,000 | 46,000,000 | 43,000,000 |
| 86 | By Brew........ | 147,000 | 4,428,000 | ........ | ........ |
| | | 157,000 | 3,933,000 | ........ | ........ |
| | | 123,000 | 3,278,000 | ........ | ........ |
| | Breed........ | 224,000 | 3,988,000 | Bd. of H. 476,000 | 492,000 |
| | | 267,000 | 4,480,000 | 493,000 | 460,000 |
| | | 220,000 | 4,128,000 | 624,000 | 493,000 |
| 87 | By Brew........ | 185,000 | 1,095,000 | ........ | ........ |
| | | 190,000 | 558,000 | ........ | ........ |
| | | 186,000 | 795,000 | ........ | ........ |
| | Breed........ | 268,000 | 1,184,000 | Bd. of H. 575,000 | 394,000 |
| | | 275,000 | 1,971,000 | 838,000 | 509,000 |
| | | 405,000 | 1,995,000 | 999,000 | 854,000 |
| 88 | By Brew........ | 121,000 | 901,000 | ........ | ........ |
| | | 104,000 | 1,018,000 | ........ | ........ |
| | | 66,000 | 959,000 | ........ | ........ |
| | Breed........ | 147,000 | 1,880,000 | Bd. of H. 345,000 | 328,000 |
| | | 74,000 | 950,000 | 345,000 | 345,000 |
| | | 107,000 | 2,725,000 | 172,000 | 312,000 |
| 89 | By Brew........ | 321,000 | 862,000 | ........ | ........ |
| | | 345,000 | 1,075,000 | ........ | ........ |
| | | 237,000 | 693,000 | ........ | ........ |
| | Breed........ | 593,000 | 1,630,000 | Bd. of H. 1,035,000 | 920,000 |
| | | 452,000 | 1,295,000 | 460,000 | 476,000 |
| | | 450,000 | 1,400,000 | 625,000 | 362,000 |
| 90 | By Brew........ | 30,000 | 294,000 | ........ | ........ |
| | | 23,000 | 241,000 | ........ | ........ |
| | Breed........ | 63,000 | 789,000 | Bd. of H. 82,000 | 115,000 |
| | | 67,000 | 542,000 | 148,000 | 131,000 |
| 91 | By Brew........ | 368,000 | 2,150,000 | ........ | ........ |
| | | 533,000 | 3,340,000 | ........ | ........ |
| | Breed........ | 485,000 | 2,922,000 | Bd. of H. 1,018,000 | 796,000 |
| | | 558,000 | 3,435,000 | 1,446,000 | 1,000,000 |
| | | 852,000 | 4,460,000 | 8,520,000 | 9,130,000 |

### General Summary of Fourth Series.

*Uniformity of results.*—The variations in the analyses of duplicate samples is about the same as in the second and third series. Somewhat widely discrepant individual variations are evident still, showing that in spite of extreme care occasional errors will be made even in the best laboratories. Among these variations it will be noticed that the laboratory D again gave the lowest numbers in practically every case. The actual relation of the laboratories to the general average and to each other is shown by the following table:

TABLE XII.

| No. | General average. | A average. | B average. | C average. | D average. |
|---|---|---|---|---|---|
| 85 | 32,000,000 | 41,000,000 | 41,000,000 | 32,000,000 | 14,000,000 |
| 86 | 348,000 | 320,000 | 244,000 | 306,000 | 122,000 |
| 87 | 377,000 | 496,000 | 450,000 | 344,000 | 147,000 |
| 88 | 293,000 | 321,000 | 495,000 | 210,000 | 149,000 |
| 89 | 304,000 | 347,000 | 176,000 | 400,000 | 149,000 |
| 90 | 134,000 | 156,000 | 145,000 | 183,000 | 54,000 |
| 91 | 1,155,000 | 1,250,000 | 1,450,000 | 1,500,000 | 426,000 |

*Comparison of the 24 and the 48 hour count.*—The 24-hour count is smaller than the 48-hour count in a majority of cases. In 52 reports the 48-hour count is the larger and in 28 cases the 24-hour count is the larger. The extent of the difference is shown by the following averages:

In the averages of the 28 series of samples (seven different bottles of milk to each laboratory) the 48-hour count was the larger in 25 cases, smaller in 1 case, and the same in 2 cases. The averages of the whole series (omitting the samples counting in millions) was 299,000 for the 48-hour count and 147,000 for the 24-hour count. In other words, on the average the 48-hour count gave about twice as large numbers as the 24-hour count. In 4 cases where the count was over 10,000,000, three times the 24 and the 48 hour count practically agreed, while in the fourth case the 24-hour count was much the lowest.

In this series of figures the test of the Detroit peptone (two other lots being furnished the laboratories) shows a slight superiority for the Witte peptone. In 45 reports the Witte gave the higher counts and in 32 the Detroit gave the higher. Given in a little more detail, the averages were as follows:

TABLE XIII.

| No. | Laboratory A. | | Laboratory B. | |
|---|---|---|---|---|
| | Standard. | Detroit. | Standard. | Detroit. |
| 85 | 44,300,000 | 29,300,000 | 45,000,000 | 36,000,000 |
| 86 | 349,000 | 294,000 | 388,000 | 245,000 |
| 87 | 549,000 | 451,000 | 510,000 | 390,000 |
| 88 | 295,000 | 348,000 | 300,000 | 386,000 |
| 89 | 346,000 | 346,000 | 193,000 | 147,000 |
| 90 | 165,000 | 148,000 | 141,000 | 149,000 |
| 91 | 1,634,000 | 1,340,000 | 1,740,000 | 1,490,000 |
| | Laboratory C. | | Laboratory D. | |
| | Standard. | Detroit. | Standard. | Detroit. |
| 85 | 31,000,000 | 33,000,000 | (?) | (?) |
| 86 | 325,000 | 287,000 | 147,000 | 199,000 |
| 87 | 333,000 | 353,000 | 170,000 | 124,000 |
| 88 | 229,000 | 192,000 | 187,000 | 163,000 |
| 89 | 484,000 | 323,000 | 317,000 | 289,000 |
| 90 | 167,000 | 132,000 | 61,000 | 50,000 |
| 91 | 1,240,000 | 1,760,000 | 44,300 | 40,700 |

Although the above figures favor the Witte peptone, the difference is very slight, and, when taken in connection with the reports on the third series of tests, is hardly sufficient to warrant regarding the one as in reality superior to the other.

The results with the dilute media were too irregular to warrant the drawing of any conclusions.

*Microscopic analyses.*—A condensed summary of the microscopic results is given in the following table. In the first column is given the average plate count of the samples submitted to Borden's laboratory, and in the next two columns the microscopic group count, both by Borden and by Brew, the representative of Breed, each based upon separate smears. In the third column is given the average plate count of the samples submitted to the Board of Health, and the next two columns give the microscopic count made by Breed and by the Board of Health on the same samples.

TABLE XIV.—*Microscopic count compared with average plate count.*

| No. | Plate. | Borden. | Brew. | Plate. | Breed. | Board of health. |
|---|---|---|---|---|---|---|
| 85 | 41,000,000 | 10,000,000 | 43,000,000 | 32,000,000 | 46,000,000 | 42,000,000 |
| 86 | 320,000 | 116,000 | 107,000 | 300,000 | 250,000 | 500,000 |
| 87 | 496,000 | 315,000 | 187,000 | 344,000 | 316,000 | 521,000 |
| 88 | 321,000 | 210,000 | 93,000 | 210,000 | 109,000 | 312,000 |
| 89 | 347,000 | 294,000 | 237,000 | 400,000 | 374,000 | 646,000 |
| 90 | 156,000 | 115,000 | 26,500 | 183,000 | 65,000 | 119,000 |
| 91 | 1,250,000 | 490,000 | 450,000 | 1,500,000 | 623,000 | 3,600,000 |

From the above table it will be seen that in general magnitude the microscopic count agrees fairly well with the plate count. The exceptions to this are no more variant from the general averages of the different laboratories than the plate-count averages of the different laboratories are from each other, as shown by the previous tables.

As in the last series, it is evident that the plate count can give no real indication of the actual number of bacteria, since the individual count is uniformly higher than the group or the plate count.

In the hands of the Board of Health laboratory the use of the wire loop for this microscopic test appears to yield results as reliable as, and practically identical with, the use of the $\frac{1}{100}$ c. c. pipette.

## GENERAL SUMMARY AND CONCLUSIONS.

1. The standard methods of Milk Analysis, published by the American Public Health Association, are in emphatic need of revision. These standard methods lay great emphasis on some of the least important points, while they neglect to lay any emphasis on some of the most important ones. The revision of these methods is now in the hands of at least three committees, one appointed by the American Public Health Association, one by the Society of American Bacteriologists, and one by the Association of Dairy Instructors.

2. Individual analyses under the best conditions are subject to considerable variation, so that no single individual count can be properly relied upon. This emphasizes the necessity of demanding an average of two or more plates in determining the bacterial content of any sample of milk.

3. The question of the exact composition of the media to be used is of far less significance than that of the methods used in the manipulation. Wide variations in the composition of the media do not make any noticeable difference in the bacterial count.[1]

4. Greater care is needed to unify laboratory methods than has hitherto been given. When the work of these four laboratories was compared at the outset it was found that there were very wide differences in the analyses of duplicate samples, due chiefly to differences in laboratory technique.

5. These variations in the analyses of duplicate samples of the same lot of milk have been found to be due to several causes:

*a*. Laboratory errors. These occasionally appear, due doubtless to confusion which is sure to arise when large numbers of samples of milk are analyzed at the same time.

*b*. Irregularities in methods of laboratory technique. These are several in number, the more important seeming to be the following:

I. Shaking of the samples. Wide variations were found in the vigor and the extent of the shaking to which the samples of milk and the dilutions are subjected by the different laboratories. While this factor does not make a very great difference in results, it is one of the irregularities that should be eliminated.

II. Amount of dilution. The counts from highly seeded plates are uniformly lower than the counts of the same milk from low-seeded plates. The best results are obtained only when the plates contain somewhere between 40 and 200 colonies. Hence the number of dilutions made in any analysis will materially affect the results.

III. Methods of counting. This has seemed to be the cause of the widest amount of irregularity. The greatest difference was associated with the use or nonuse of a counting lens, or to differences in magnifying power of the lens used.[2]

Even when this difference is eliminated the further results show that the personal equation in counting is still a factor of very large importance. When the same plate is counted by two different laboratories the results are not infrequently 100 per cent apart, and occasionally even more.

A third factor modifying the counting is the method adopted by each laboratory of estimating numbers rather than actual counting. In plates that contain large numbers of colonies it is of course necessary to make an estimate rather than to count them all. The laboratories adopt different methods in such conditions, the results being slightly different.

---

[1] For this reason and in order to produce uniformity, there has already been adopted by the American Public Health Association a change in the standard methods by which beef extract can be substituted for beef infusion and the acidity of the standard agar fixed at 1 per cent, thus making it uniform with that of the standard used in water analyses.

[2] The recognition of this fact has already led to a modifying of the standard methods, which now require a lens of 3½ diameters to be used in counting all plates.

IV. Irregularities in samples from the same bottle of milk. Two samples taken from the same bottle of milk, even after thorough shaking, are by no means identical. This is easily explained, and is due (1) to the clumping of bacteria and (2) to the fact that inasmuch as bacteria are not in solution but are solid objects they can not be expected to be uniformly distributed through the liquid. The tests show that one sample of a bottle of milk when tested by the plate method may sometimes not contain more than one-fourth as many bacteria as other samples from the same bottle.

V. In low counts variation between duplicate samples is sometimes considerable, due to the irregularity of the distribution of bacteria.

In high counts variation in reports is also sometimes very great, due to excessive crowding and to methods adopted in estimating the number of colonies.

6. The extent of the variation in the results obtained from the analysis of duplicate samples varies widely with the care that is taken in the laboratory technique. It has been found in these tests that at first the above causes of irregularity were sufficient to give results disagreeing as much as tenfold in the number of bacteria that would be reported from any sample of milk. The vast majority of results, however, were much closer than this, even at the beginning of this series of tests.

7. After attention had been called to the points of irregularity and the laboratories had adopted methods of bringing about uniformity in technique so far as possible, the variations were very greatly reduced, the last tests showing that when sufficient care is given the variations need not be more than twofold. It is not possible to rely upon a greater accuracy than 100 per cent even when an average of more than one analysis is obtained, although most of the results fall considerably below this limit.

8. There is no essential difference in the results whether milk dilution is directly inoculated into the petri dish and the agar poured upon it, or whether the milk dilution is inoculated into the melted tube of agar and subsequently poured into the petri dish. The difference between the two methods is so slight as to make it impossible to determine which is the superior of the two. But when examinations of cream are made the plate inoculation is unreliable, and the inoculation must be made in the agar tube followed by thorough agitation.

9. Five days' incubation (48 hours at 37° and 72 hours at 20°) gives a very slightly greater count than a two-day incubation. The increase in count is so slight that for general regulation purposes it is hardly superior to a two-day count; and considering the superior value of obtaining the count promptly there seems to be no reason for changing from a two-day count to a five-day count.

10. A 24-hour count gives on the average about one-half as high numbers as a 48-hour count.

11. In spite of all these irregularities the results with duplicate samples in the four laboratories have been found, within certain somewhat wide limits, fairly accurate. They are at all events accurate enough to warrant three broad grades, essentially three grades that have been adopted by the commission on milk standards. But they are not as yet accurate enough to warrant a closer grading than the commission's grades A, B, and C, A including all below 200,000 (or 100,000), B from 200,000 to 1,000,000, and C including all above 1,000,000. For this broad grading it is necessary to have an average of at least four or five separate analyses in order to rely upon the results. Even then there will be an occasional overlapping of grade B with either grade C on the one hand or grade A on the other.

12. This series of tests has proved that if a sample of milk can be put into iced water, containing floating ice, it may be kept for 20 hours with very little change in bacteria count. This makes it possible to keep samples sent to a laboratory for analysis for a number of hours without any fear of change in bacterial content, provided the samples are immersed in water containing floating ice.

13. These tests have seemed to indicate that the American peptone, made by the Digestive Ferment Co., of Detroit, can be substituted for Witte peptone without materially changing the results.

14. *Direct microscopic method of bacterial examination of milk by the Breed method.*—In making a comparison of the bacteriological analysis by the plate count and the microscopic count, the latter should be a count of groups rather than individuals, plate colonies representing groups only.

15. Considerable experience by the person making the count is needed to distinguish between bacteria and dirt particles, particularly when the milk contains minute micrococci.

16. When the microscopic count is made by one who has had sufficient experience, the group count agrees somewhat closely with the plate count—agreeing, indeed, about as closely as the plate counts of different laboratories agree with each other.

17. Raw, fresh milk does not contain any appreciable number of dead bacteria which might disclose themselves to the microscope, but fail to grow in plates.

18. The direct microscopical examination of milk smears by the Breed method will classify raw milk into grades A, B, and C with about the same accuracy and much more quickly than the plate method of bacteriological analysis will do. It is of no use in the study of pasteurized milk, however, since it discloses dead as well as living bacteria, no method of distinguishing between them having

yet been perfected. It might be of value in telling whether such milk had become old before it was pasteurized, since such would show large numbers of dead bacteria by the microscopic method, though it might show small numbers by the plate method.

19. The direct microscopical method of bacteriological analysis might be of exceptional value applied at the dairy to guide the dairyman as to the general grade of the milk he is marketing. It may also be of great aid to the large dealer to enable him to determine promptly whether he is purchasing milk of A, B, or C grade. The possibility of quick results and ease of making the smears at the dairy or shipping station, subsequently sending them to the laboratory for microscopic examination, renders the method especially applicable at the dairy end of the line.

## PLAGUE-PREVENTION WORK.

### CALIFORNIA.

The following report of plague-prevention work in California for the week ended July 24, 1915, was received from Passed Asst. Surg. Hurley, of the United States Public Health Service, in temporary charge of the work:

### SAN FRANCISCO, CAL.

#### RAT PROOFING.

| | |
|---|---|
| New buildings: | |
| Inspections of work under construction | 213 |
| Basements concreted (square feet, 18,056) | 25 |
| Floors concreted (square feet, 4,190) | 7 |
| Yards, passageways, etc. (square feet, 8,470) | 53 |
| Total area of concrete laid, square feet | 30,716 |
| Class A, B, and C (fireproof) buildings: | |
| Inspections made | 208 |
| Roof and basement ventilators, etc., screened | 5,205 |
| Wire screening used, square feet | 25,410 |
| Openings around pipes, etc., closed with cement | 8,593 |
| Sidewalk lens lights replaced | 1,540 |
| Old buildings: | |
| Inspections made | 241 |
| Wooden floors removed | 15 |
| Yards and passageways, planking removed | 4 |
| Cubic feet new foundation walls installed | 3,055 |
| Concrete floors installed (square feet, 42,775) | 36 |
| Basements concreted (square feet, 7,370) | 15 |
| Yards and passageways, etc., concreted (square feet, 13,638) | 38 |
| Total area concrete laid, square feet | 63,783 |
| Floors rat proofed with wire cloth (square feet, 2,516) | 6 |
| Buildings razed | 9 |
| New garbage cans stamped approved | 722 |
| Nuisances abated | 213 |

#### OPERATIONS ON THE WATER FRONT.

| | |
|---|---|
| Vessels inspected for rat guards (times) | 22 |
| Reinspections made on vessels | 28 |
| New rat guards procured | 8 |
| Defective rat guards repaired | 10 |
| Vessels on which cargo was inspected | 1 |

| Amount of cargo and description of same. | Condition. | Rat evidence. |
|---|---|---|
| Steamer President from Seattle: | | |
| 162 cases salmon, candy, ham, and household goods | O. K. | None. |
| 300 sacks flour and rice | O. K. | None. |